

30 March 2012 | \$10

Science



 AAAS

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COVER

Two *Daphnia dentifera*, each ~2 millimeters long. The organism at bottom left is infected with a virulent yeast parasite, causing her bright white appearance. The food web in which the host-parasite interaction is embedded determines the size of the epidemic, which, in turn, determines whether the *Daphnia* population becomes more resistant or more susceptible during the epidemic. See page 1636.

Credit: Meghan A. Duffy

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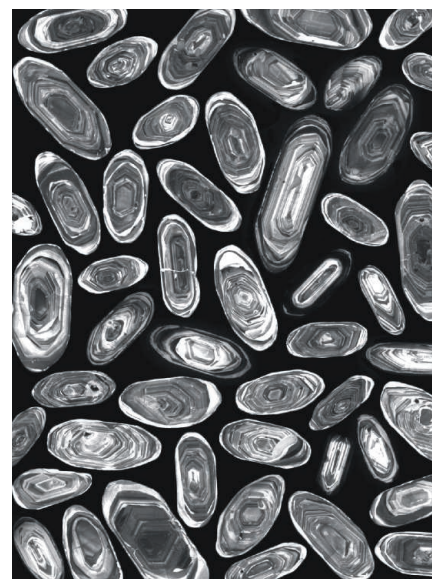
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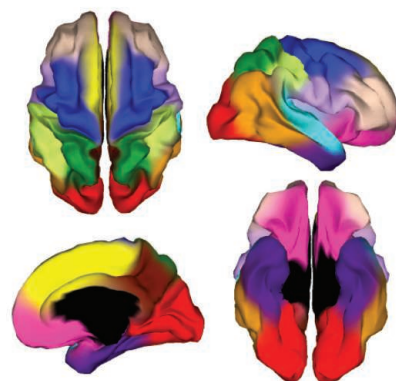
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Bumble bee colonies produce many fewer queens after exposure to a widely used insecticide.
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M. Henry et al.

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A. R. Shenoy et al.

A human protein activates the assembly of a cellular complex that detects signs of infection.
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A 2010 disaster coated deep-sea corals with oil more than 11 kilometers from the well site.
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Findings Cast Doubt on Moon Origins

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SCIENCE SIGNALING

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The Signal Transduction Knowledge Environment

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Aberrant activation of mTORC1 disrupts tissue homeostasis, leading to carcinogenesis.

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28 March issue: <http://scim.ag/stm032812>

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T. Zhen et al.

SCIENCE CAREERS

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Does a Professional Science Master's Degree Pay Off?

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E. Pain

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NPA Founders Find Success

M. Price

The founders the National Postdoctoral Association say that the experience helped their careers.

http://scim.ag/NPA_Founders

SCIENCEPODCAST

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Free Weekly Show

On the 30 March Science Podcast: uranium age-dating, the brain's geometry, data replication, and more.

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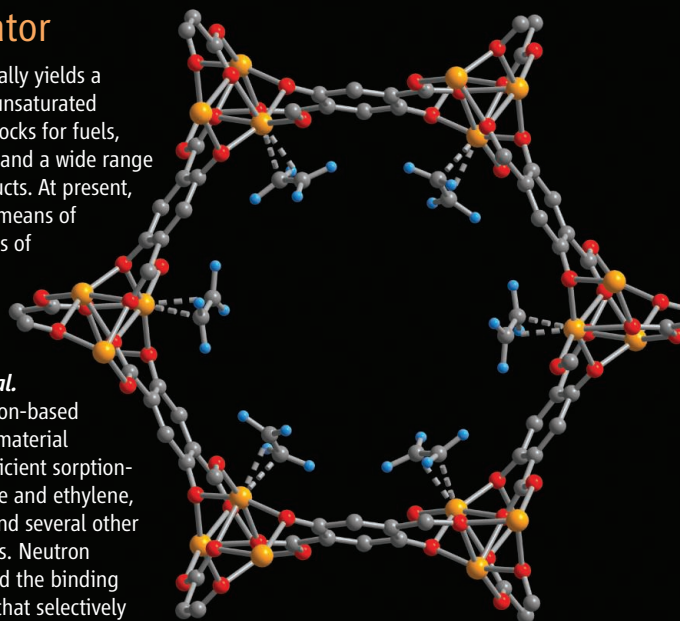
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ADVANCING SCIENCE. SERVING SOCIETY

An Iron Separator

Petroleum processing initially yields a mixture of saturated and unsaturated hydrocarbons—the feedstocks for fuels, plastics, pharmaceuticals, and a wide range of other commercial products. At present, distillation is the primary means of separating the components of this mixture. A sorbent or membrane-based approach to separation could reap substantial energy savings. **Bloch *et al.*** (p. 1606) found that an iron-based metal organic framework material shows promise for very efficient sorption-based separation of ethane and ethylene, propane and propylene, and several other light hydrocarbon mixtures. Neutron diffraction directly revealed the binding motifs at the iron centers that selectively pinned down the olefins while the saturated hydrocarbons passed by.



Chiral Ice

Water ice, even at the lowest temperatures, is not completely “frozen”—its lattice structure allows for multiple equivalent ground states and it thus retains finite entropy even at absolute zero. Equivalent structures are realized in frustrated magnets called spin ices, where spins interact ferromagnetically, and in the even more exotic artificial spin ices, which are fabricated arrays of nanoscale magnets. **Branford *et al.*** (p. 1597) studied the transport behavior of an artificial spin ice with a honeycomb geometry during upward and downward sweeps of an external magnetic field, which revealed a field-asymmetric peak when the magnetic field was applied parallel to the current and the voltage was measured transversely. Micromagnetic simulations suggest that the asymmetric response is a result of the loops of opposite handedness forming at the edges of the sample, resulting in an overall chirality of the transport response.

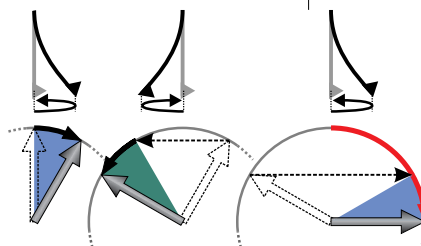
Electrons Beat Phonons

The phenomenon of superconductivity, in which a material suddenly (below a certain transition temperature T_c) becomes a perfect conductor with zero electrical resistance, can be roughly explained in terms of Bose-Einstein condensation of pairs of electrons. In conventional superconductors, the formation of these so-called Cooper pairs is mediated by lattice deformations

(phonons), but this mechanism is insufficient to explain the high T_c of cuprate superconductors. Other mechanisms, such as magnetic fluctuations, have been proposed which originate with the electrons themselves rather than the lattice. **Dal Conte *et al.*** (p. 1600) used time-resolved optical spectroscopy of an optimally doped cuprate to show that the temporal evolution of the reflectivity is consistent with the electronic contribution being dominant and is able to account for the high T_c by itself.

Quantum Mechanical Coupling

Observing the induced patterns of iron filings as a magnet is moved nearby, is a mainstay experiment of elementary science kits. Scaling down to the motion of the magnet and the size of the “sensing” particles enters the realm of quantum nanomechanics, where the motion of the vibrating system is quantized. That motion, however, is difficult to observe and manipulate. **Kolkowitz *et al.*** (p. 1603, published online 23 February; see the Perspective by **Treutlein**) coupled the single-mode vibration of a magne-



net coupled the single-mode vibration of a magne-

tized nanomechanical resonator to the quantum mechanical two-level spin system associated with the nitrogen vacancy center in diamond. The evolution of the spin degree of freedom was directly mapped to the mechanical motion, providing the opportunity to probe minute mechanical motion that would otherwise be undetectable.

A New Lease on Half-Life

Radiometric dating relies on measuring the abundance of long-lived radionuclides relative to the abundance of their radiogenic decay products—a process determined by the original radionuclide’s half-life. For primordial radionuclides that decay slowly, such as ^{146}Sm decaying to ^{142}Nd , this method provides the timing of some of the earliest processes in solar system history. Using accelerator mass spectrometry, **Kinoshita *et al.*** (p. 1614) provide a revised estimate for the ^{146}Sm half-life of ~68.7 million years, which is 30% shorter than the previously accepted value. This shorter half-life suggests that reductions need to be made in the estimated ages for differentiation of Earth’s mantle and the solidification of the Moon’s magma ocean and for other more recent processes.

Making the CoTC

In eukaryotes, cotranscriptional cleavage (CoTC) of nascent RNA transcripts at the polyadenylation (polyA) site is involved in transcription termination of RNA polymerase II genes. The Dicer endoribonucleases, on the other hand, are generally associated with gene silencing through the generation of small RNAs from double-stranded RNA. Now, **Liu *et al.*** (p. 1621) show that transcriptional read-through of the *Arabidopsis FCA* gene is regulated by *DICER-LIKE 4 (DCL4)*. DCL4 associated with the *FCA* gene downstream of the polyA site and repressed transcriptional read-through. The C terminus of DCL4 has a similar domain structure to the yeast endoribonuclease Rnt1, involved in CoTC, which suggests that DCL4 is involved in repressing read-through of endogenous *FCA* by prompting cotranscriptional cleavage.

Spreading the Excitation

Inhibitory interneurons balance network excitation, control spike-time precision of principal neurons, and control synchrony within and across brain regions. **Vervaeke *et al.*** (p. 1624, published online 8 March) combined electro-

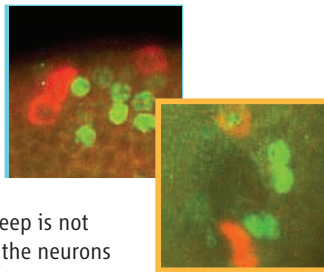
physiology, immunohistology, and numerical simulation to investigate the properties of Golgi cells, the main inhibitory interneurons in the input layer of the cerebellar cortex. The dendrites of these neurons were truly passive and acted as linear cables. Gap junctions were distributed nonuniformly on the Golgi cell surface, with a higher density on distal dendrites. Thus, gap junction-mediated lateral excitation preferentially enhanced the distal inputs, enabling distal synapses to drive network activity more effectively.

Building the Brain

Brain connectivity is often described as a network of discrete independent cables analogous to a switchboard, but how is the physical structure of the brain constructed (see the Perspective by **Zilles and Amunts**)? **Wedeen et al.** (p. 1628) used high-resolution diffusion tensor imaging in humans and four species of nonhuman primates to identify and compare the geometric structure of large fiber tracts in the brain. Fiber tracts followed a highly constrained and regular geometry, which may provide an efficient solution for pathfinding during ontogenetic development. Much of development occurs through elaboration and assembly of semiautonomous building blocks. **Chen et al.** (p. 1634) applied statistical analysis to the form of the human cortex in brain-imaging studies that compared more than 400 di- and mono-zygotic twins. The findings suggest that the structure of the human cortex is defined by genetics.

Sleeping Around the Clock

Fruit flies do not sleep all night like humans, but they do manage to spend about half of their day in bouts of inactivity that have similarity to sleep in other species. **Rogulja and Young** (p. 1617) searched for genes in neurons whose products regulated sleep. Depletion of the protein regulator of cyclin A1 (*Rca1*) caused the flies to be slow to go to sleep and to get less total sleep in a day because their sleep episodes were shorter. The mechanism by which *Rca1* influences sleep is not yet clear. It appears not to disrupt circadian rhythms. However, the neurons that express *Rca1* in the fly brain are located near the neurons that make up the circadian clock, potentially allowing for coordination of circadian behavior with sleep regulation.



Cost-Benefit Analysis

Mounting resistance to infection is costly, requires energetic input, and may thus compromise fecundity. **Duffy et al.** (p. 1636; see the cover) tested the relationships between productivity, predation, and mortality in a combination of observations of a natural lake and an experimental replica of a clonal zooplankton-parasitic yeast population. In the wild, epidemics of the yeast could exceed 60% and cause significant host mortality. In this situation, the clonal zooplankton host faces the physiological dilemma of either increasing resistance to deal with infection or of safeguarding fecundity. Zooplankton that feed quickly can reproduce quickly, but also end up ingesting more yeast spores. However, because fish tend to cull infected hosts, fish predation counters infection. Ultimately, both wild and model systems showed that lakes with high productivity (more nitrogen) and/or few fish supported greater epidemics of yeast and more resistant hosts, whereas less productive lakes, or those with more fish, had smaller epidemics and hosts with higher susceptibility to the yeast.

Dissecting Rapamycin Responses

Long-term treatment of mice and other organisms with the drug rapamycin extends life span. But, at the same time, the drug disrupts metabolic regulation and the action of the hormone insulin. **Lamming et al.** (p. 1638; see the Perspective by **Hughes and Kennedy**) dissected the action of rapamycin in genetically modified mice and found, encouragingly, that these two actions of rapamycin can be separated. Rapamycin inhibits a protein kinase complex known as mTORC1, and this appears to provide most of the life-lengthening effects of the drug. However, rapamycin also acts on a related complex known as mTORC2, and it is the disruption of mTORC2 action that produces the diabetic-like symptoms of decreased glucose tolerance and insensitivity to insulin.

CREDIT: ROGULJA AND YOUNG

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Engage to Excel

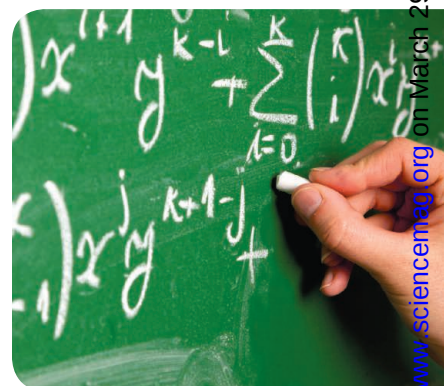
IN 2010, WE AND OUR COLLEAGUES ON THE PRESIDENT'S COUNCIL OF ADVISORS ON SCIENCE and Technology (PCAST) released a report entitled *Prepare and Inspire: K-12 Education in Science, Technology, Engineering and Math (STEM) Education for America's Future*. This important report advocates preparing all students to use STEM in their personal and professional lives and inspiring them to learn STEM subjects and pursue STEM careers. But in the United States, over 60% of students who enter college intending to major in a STEM field fail to graduate with a STEM degree. Because economic analyses forecast that the United States will need 1 million more STEM graduates over the next decade than will be produced by our current modes of education,* reducing this dropout rate is the focus of a second report we released last month, called *Engage to Excel: Producing One Million Additional College Graduates with Degrees in Science, Technology, Engineering, and Mathematics*.†

Why do undergraduate students leave STEM during the first 2 years? Studies indicate three primary reasons: uninspiring introductory courses, difficulty with the required math, and an academic culture in STEM fields that is often unwelcoming. These problems can be especially severe for members of groups underrepresented in STEM fields, including women and minorities, who today constitute about 70% of college students but earn only 45% of STEM degrees.

Engage to Excel recommends that the federal government catalyze widespread adoption of empirically validated teaching practices, including active learning approaches using case studies, problem-based learning, peer instruction, and computer simulations. Classroom approaches that engage students actively increase retention of information, build critical thinking skills, induce more positive attitudes toward STEM disciplines, and strengthen retention of students in STEM majors. In one study, students learned twice as much in a large physics course taught with active learning techniques than did students taught in a parallel class by lecture.‡

The current system delays the hands-on research and internship experiences that capture the thrill of inquiry and discovery for STEM majors until the third and fourth years of college, when many students have already opted out. One study found that college sophomores who engaged in research projects with a professor were much less likely to leave STEM majors than those who did not.§ The government should therefore advocate and provide support for replacing standard laboratory courses with discovery-based research courses. Too often, even the active learning elements of today's teaching regimens—laboratory courses—simply repeat classical experiments rather than engage students in compelling experiments with the possibility and excitement of true discovery. Examples of good alternatives can be found among the winners of *Science's* IBI Prize, as represented on pp. 1588 and 1590 of this issue. The government should also launch a national experiment in postsecondary mathematics education to address the mathematics preparation gap. Nearly 60% of students enter college without the mathematics skills needed for STEM majors. This not only limits students' ability to enter STEM careers, but costs a great deal: Colleges spend approximately \$2 billion per year on developmental education.

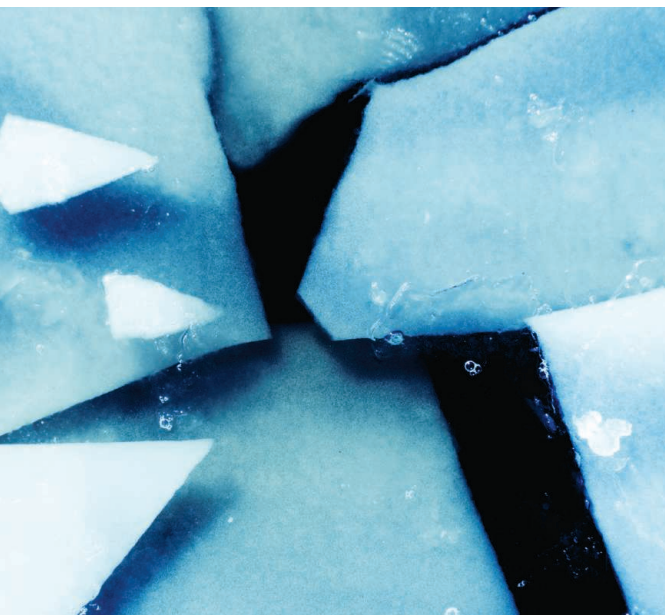
PCAST's recommendations, many of which could be implemented by refocusing current STEM investments, address the most substantial barriers to STEM student retention and have the potential to catalyze change in America's college classrooms. Their implementation will give students the skills they need to fill 21st-century jobs and give the United States the workforce it needs to be innovative and competitive. — S. James Gates Jr. and Chad Mirkin



10.1126/science.1222058

*D. Langdon, G. McKittrick, D. Beede, B. Khan, M. Doms, *ESA Issue Brief* 03-11 (Economics and Statistics Administration, U.S. Department of Commerce, Washington, DC, 2011). †http://www.whitehouse.gov/sites/default/files/microsites/ostp/pcast-engage-to-excel-final_2-25-12.pdf. ‡L. Deslauriers, E. Schelew, C. Wieman, *Science* **332**, 862 (2011).

§B. A. Nagda, S. R. Gregerman, J. Jonides, W. von Hippel, J. S. Lerner, *Rev. Higher Educ.* **22**, 55 (1998).



CLIMATE SCIENCE

Extreme Melting

Climate models have predicted that extreme weather events will increase in frequency and intensity as greenhouse gases continue to accumulate in the atmosphere and the consequent global warming intensifies. Such a change could have tremendous practical impacts on our environment and our lives. It is of interest, therefore, to understand the mechanisms that may cause this phenomenon. One obvious part of the world toward which to look for evidence is the Arctic, where the rate of warming has been faster than anywhere else on Earth. Francis and Vavrus examined atmospheric data from the mid- and high-latitude Northern Hemisphere and identified two mechanisms that seem to contribute to the trend toward extremes, both of which have the effect of slowing down the west-to-east progression of weather systems in the northern mid-latitudes, thereby intensifying their impacts. These changes seem to be the result of growing Arctic sea-ice loss, which causes the transfer of additional energy from the ocean into the high-latitude atmosphere, and of earlier continental snow melt and drying of the soil, which increase the tendency toward high-amplitude weather patterns in summer. — HJS

Geophys. Res. Lett. **39**, L06801 (2012).

CHEMISTRY

Golden Proteins

Gold nanoparticles (Au NPs) have long been used as an aid in biological imaging, in part because of their low chemical reactivity. Malay *et al.* studied the effect of covalent attachment of Au NPs to TRAP (*trp* RNA-binding attenuation protein), which plays a feedback role in tryptophan biosynthesis in *Bacillus*, by mutating a Lys residue to Cys. The TRAP protein normally forms a ring of 11 monomers and has been of interest for the delivery of nanoscale objects. However, the incubation of the Cys-bearing protein with 1.4-nm Au NPs created two types of structures similar to viral capsids: hollow shells either 15 to 16 or 21 to 22 nm in diameter. These structures did not form when the native protein was simply mixed with Au NPs, and their formation depended on pH conditions and the concentration of Au NPs. Cryoelectron microscopy also revealed that not all of the capsids retained the Au NPs, which suggests that their role may be to catalyze the rearrangement from the toroidal structure. — PDS

Nano Lett. **10**, 1021/nl3002155 (2012).

BIOMEDICINE

Ovarian Cancer Origins

One to 2% of women will be diagnosed with ovarian cancer in their lifetimes. As a result, there is great interest in better understanding the molecular and cellular events that drive the development of this cancer. In fact, whether the ovary or fallopian tube gives rise to serous ovar-

ian cancer, the subtype that causes 70% of ovarian cancer deaths, is still unknown. To investigate this question, Kim *et al.* generated mice with reproductive tract-specific deletions in *Dicer*, the enzyme that converts pre-microRNAs to mature microRNAs, and *Pten*, a tumor suppressor. They found that serous ovarian carcinomas developed from the fallopian tube and then metastasized elsewhere, killing all double knockout mice by 6 to 12 months of age. Mice singly deficient in these proteins, however, did not show tumor development in the reproductive system. Histological analysis revealed that abnormal cell proliferation started in the stromal compartment of the fallopian tube rather than the epithelial layer, with the cells undergoing a stromal-to-epithelial transition. The phenotypic, histological, and molecular characteristics of the cancers that developed in the double knockout mice were similar to those seen in humans. These mice may therefore provide a useful model system for studying serous ovarian cancer. — BAP

Proc. Natl. Acad. Sci. U.S.A. **109**, 3921 (2012).

CELL BIOLOGY

Knocking on Cilia's Door

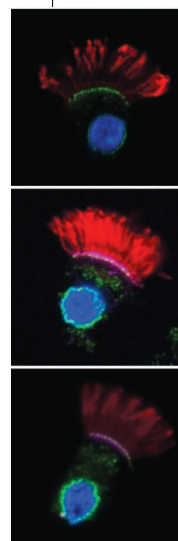
Primary cilia are hairlike projections found at the surface of differentiated cells that play a role in development and signalling. Defects in ciliary protein localization and function are linked to human diseases, including retinal

degeneration, polycystic kidney diseases, skeletal defects, and obesity. Microtubules provide cilia with their fundamental structural integrity, but cilia contain a multitude of specific proteins and are ensheathed by a specialized membrane.

The entry of proteins from the cytosol into the cilium appears to be restricted at its base, and there is evidence that components of the nuclear import machinery may play a role. Kee *et al.* exploited the potential similarities between nuclear import and ciliary protein import to try to dissect the molecular mechanisms involved. Like the nuclear pore, the base of the cilium in mammalian tissue culture cells excluded microinjected proteins larger than ~40 kD, whereas smaller

proteins freely diffused into the cilium. This diffusion barrier appeared to be related to the presence of nucleoporins, some of which could be localized at the base of the cilium. Indeed, microinjection of nucleoporin function-blocking agents restricted the entry of a ciliary motor protein. Thus, the base of the cilium appears to play a role similar to that of the nuclear pore in restricting access. How ciliary proteins are then specifically allowed to enter, however, remains unclear. — SMH

Nat. Cell Biol. **14**, 10.1038/ncb2450 (2012).



CREDITS (TOP TO BOTTOM): ISTOCKPHOTO.COM; NAT. CELL BIOL. **14**, 10.1038/ncb2450 (2012)

ECONOMICS

Not So Committed?

What factors influence the willingness of companies to engage in acts of social responsibility, and when does it represent a real commitment rather than window dressing? To study this question, Lim and Tsutsui looked at two global initiatives. The United Nations Global Compact encourages businesses to use sustainable practices and focuses on human rights, labor, the environment, and anticorruption. Governments can formally endorse the program, and corporations who participate are expected to submit annual Communications of Progress. The Global Reporting Initiative, which provides standards and Sustainability Reporting Guidelines, requires an even more substantial commitment. Using data from 99 countries spanning 2000–2007, the authors found that nongovernmental organization linkages encouraged the adoption of corporate social responsibility policies. In developing countries, this was associated with substantial commitment; however, in developed countries, commitment was more ceremonial. Liberal economic policies in the developed world were also associated with ceremonial commitment, suggesting to the authors a “pattern of organized hypocrisy” in which costly norms are imposed by the developed world on others. — BJ

Am. Sociol. Rev. **77**, 69 (2012).

CHEMISTRY

More Oomph for OPH

Enzymes are remarkably adept at accelerating complex chemical reactions, but they tend to work best in the milieu where they evolved. It is therefore often challenging to apply them at high temperature or in the midst of interfering factors. El-Boubbou *et al.* sought to optimize the use of organophosphorus hydrolase (OPH) in the service of degrading the highly toxic organophosphorus compounds present in certain pesticides and nerve agents. They found that immobilization of the enzyme in mesoporous silica prefunctionalized with ammonium ions

conferred substantial thermal stability without compromising the flexibility necessary for catalytic activity. Phosphorus-31 nuclear magnetic resonance spectroscopy was applied to monitor rates for hydrolysis of the model compound paraoxon, and revealed enhanced specific activity of the immobilized enzyme relative to the native enzyme in solution. The silica-bound enzyme remained active after being heated (either dry or in suspension) to temperatures as high as 65°C for extended periods—as long as a month at 45°C. — JSY

Adv. Healthcare Mater. **1**, 183 (2012).

MICROBIOLOGY

In Need of Nutrients

Gut pathogens such as *E. coli* and *Salmonella* are faced with several hurdles when trying to

establish an infection: the recruitment of immune cells, the secretion of antimicrobial factors, and competition in the form of the billions of commensal bacteria that normally reside in our guts. As a result, the pathogens need to have a few tricks up their sleeve.



Liu *et al.* now report on one such example, used by *Salmonella enterica* serovar Typhimurium, a pathogen that causes severe gastroenteritis in humans. In response to *S. Typhimurium* infection, neutrophils are recruited to the gut in mice and produce the antimicrobial protein calprotectin. Calprotectin functions by sequestering essential metals, such as zinc, thus limiting an important nutrient source for the invading pathogen. *S. Typhimurium* can overcome this, and compete with the commensal flora, however, because it expresses a high-affinity zinc transporter. Strains that lacked this transporter did not grow as well in the inflamed gut but were not at a disadvantage in the absence of inflammation. These results suggest that nutrient availability is a key factor in the establishment of gastrointestinal infections. — KLM

Cell Host Microbe **11**, 227 (2012).

105

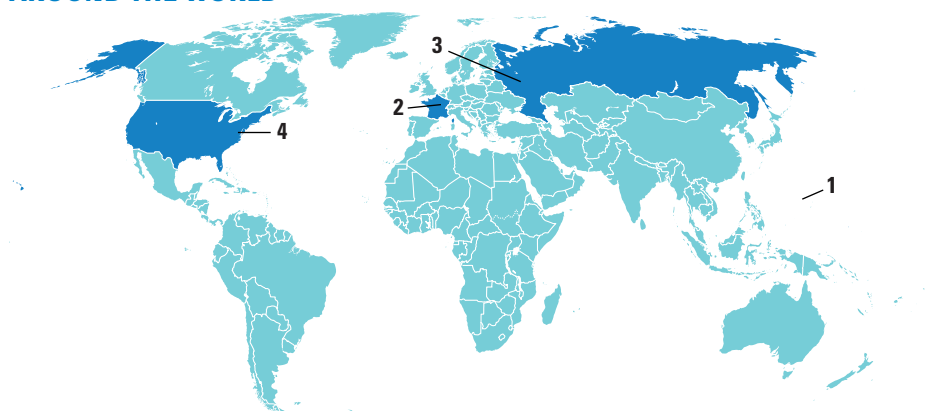
things you didn't
(and 3 you probably
shouldn't) know
about some of
your most
respected
colleagues.

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AROUND THE WORLD



Mariana Trench, Western Pacific Ocean 1

World's First Solo Dive To Challenger Deep

On the morning of 26 March, movie director James Cameron completed the world's first solo dive to the deepest part of the world's oceans—an 11-kilometer-deep valley within the Mariana Trench known as Challenger Deep, 322 kilometers southwest of Guam. Cameron's submersible *Deepsea Challenger* was outfitted with biological and sediment sampling equipment; temperature, salinity, and pressure sensors; and still and video cameras to document the historic dive.



Deep dive. James Cameron reached Challenger Deep on 26 March.

It took Cameron 2.5 hours to reach the bottom, but only about 70 minutes to come back up. Leaking hydraulic fluid cut the planned 6-hour dive in half, and Cameron was only able to retrieve a partial sediment core from this lonely seascape. He reported an absence of the large jellyfish and anemones he saw during a test dive in the New Britain Trench off Papua New Guinea. Challenger Deep's fine-silt bottom, he noted, was devoid of any animal tracks.

Cameron's descent into Challenger Deep marks the second such manned attempt after late Swiss engineer Jacques Piccard and retired U.S. Navy Captain Don Walsh touched down in their bathyscaphe *Trieste* in 1960.

Strasbourg, France 2

Leading French Institute Director Ousted

A long-running dispute at one of France's leading biology institutes has culminated in the removal of the director. Developmental biologist Olivier Pourquié, who has led the Institute of Genetics and Molecular and Cellular Biology (IGBMC) in Strasbourg since 2009, was informed last month that his term as director would end on 1 June. The decision to remove Pourquié—taken by the presidents of the institute's funders, CNRS, INSERM, and the University of Strasbourg—follows months of internal rancor, including anonymous e-mails, libel accusations, and attempts to calm the waters with an external mediator.

Pourquié says he tried to bring transparency and a modern governance structure to the institute, but was blocked by a subset of researchers who felt threatened by the changes.

"Pourquié is a scientific star. This was why he was chosen," says Iain Mattaj, director general of the European Molecular Biology Laboratory in Heidelberg, Germany, who is head of IGBMC's scientific advisory board. But "he had no leadership experience and little experience of the French system." The institute lacked rules for governing itself, he adds, and "putting [those rules] in place is essential prior to appointing a successor."

<http://scim.ag/Pourquie>



Crashed. Launched 18 August, Russia's Express-AM4 satellite came back to Earth 25 March.

Moscow 3

No Second Life for Russian Satellite

Russian engineers intentionally crashed the telecommunications satellite Express-AM4 on 25 March, despite 11th-hour pleas from a company hoping to salvage the spacecraft for Antarctic communications.

The satellite launched 18 August, but mechanical failures left it in a too-low and useless orbit. The satellite was still functional, but the Russian Satellite Communications Company's chief financial officer, Dennis Pivnyuk, said earlier this month at the Satellite 2012 conference in Washington, D.C., that the Russian government had considered—and rejected—numerous salvage plans.

Polar Broadband Systems Ltd. had proposed one of those plans, crafting a new orbit for the satellite that they said would provide 16 hours of coverage to the National Science Foundation's (NSF's) South Pole Station. Although they said NSF expressed interest, the company was ultimately unable to obtain the spacecraft from the Russian State Commission.

Engineers at Astrium, which designed the spacecraft, fired its engines to begin a controlled descent at 6:33 a.m. EDT on Sunday. The debris landed a few hours later in the northern Pacific Ocean.

Washington, D.C. 4

Last Days for Cancer Gene Patents?

In the past 10 days, the U.S. Supreme Court has delivered a subtle, two-part blow that cuts the ground from under some hotly contested biotech patents, including those on breast and ovarian cancer genes *BRCA1* and *BRCA2*. On 20 March, the court voted

unanimously to reject patents for a test to monitor the anti-inflammatory drug azathioprine, held by Prometheus Laboratories of San Diego, California. The test, according to the opinion penned by Justice Stephen Breyer, was based on standard human responses that are like laws of nature—and laws of nature cannot be patented. The second blow came on 26 March when the court issued a terse announcement saying it would “vacate,” or dismiss, a decision by a lower court that upheld the validity of the *BRCA1* and *BRCA2* patents. The Supreme Court sent the case back down to the Court of Appeals for the Federal Circuit in Washington, D.C., “for further consideration” in light of the Prometheus decision. Biotech patent expert Christopher Holman of the University of Missouri, Kansas City, School of Law says the justices may want the lower court to knock out those gene patents. <http://scim.ag/genepats>

NEWSMAKERS

Endre Szemerédi Wins Math's Biggest Prize

Endre Szemerédi of Rutgers University in New Brunswick, New Jersey, and the Alfréd Rényi Institute of Mathematics in Hungary, has received the 2012 Abel Prize for mathematics, “for his fundamental contributions to discrete mathematics and theoretical computer science,” according to a prize committee announcement made 21 March. The 6 million kroner (\$1 million) prize has been given out annually by the Norwegian Academy of Science and Letters since 2003.



Szemerédi

Szemerédi's work, mainly in combinatorics (the study of arrangements of finite systems) and number theory, has shown that, as systems made up of discrete components—such as hyperlinked pages on the World Wide Web—get large, there is structure even when the system is utterly random.

His most famous result is an eponymous theorem establishing the presence of arbitrarily long arithmetic progressions, the epitome of orderliness, within any sequence of integers that doesn't become infinitely sparse. The paper it appeared in “is a real tour de force,” says Terence Tao, a mathematician at the University of California, Los Angeles, who was on the committee that chose this year's winner. <http://scim.ag/Szemerédi>



Frozen in Time

Earth scientists aren't going to let a unique collection of glacier photographs melt away. This month, glaciologist Matt Nolan of the University of Alaska, Fairbanks, won a grant from the National Science Foundation to start digitizing and preserving about 100,000 large-format aerial photographs of glaciers in Alaska, Canada, and Washington state taken between 1960 and 1995. “It is quite simply the best and most comprehensive record of these glaciers over that time span,” says Nolan. “It was the satellite data of its time.”

Most of the negatives were made by Austin Post, a photographer legendary among ice scientists for his sharp eye and insight. The now-retired Post never got a college degree, but in 2004 was awarded an honorary doctorate for his work, mostly done for the U.S. Geological Survey. Ultimately, Nolan hopes to revisit the glaciers documented by Post—such as this glacier near Summit Lake, Alaska, shown above in 1970—and shoot updated portraits to see how the rivers of ice have changed.

Obama Nominates Dartmouth President to Head World Bank

President Barack Obama surprised many people on 23 March by nominating Dartmouth College president and global health expert **Jim Yong Kim** to head the World Bank. “It's time for a development professional to lead the world's largest development agency,” said Obama in a speech at the White House's Rose Garden.

Kim is a physician and anthropologist who co-founded Partners in Health with Paul Farmer and later headed the HIV/AIDS program at the World Health Organization (WHO). His work with Partners in Health included launching a pioneering program for treating multidrug-resistant



Kim

tuberculosis in Peru. At WHO, he led a program called 3 by 5 that pushed for the goal of having 3 million HIV-infected people on antiretroviral drugs by 2005. The project did not hit its target, but more than

6 million people now have access to those lifesaving drugs.

The World Bank is expected to elect a new president when it holds a meeting on 21 April. The United States traditionally has selected presidents for the bank, but there has been an increasing call for transparency, and Kim's nomination does not guarantee him the job. <http://scim.ag/jimyongkim>

FINDINGS

One Drug to Shrink All Tumors

A single drug can shrink human breast, ovary, colon, bladder, brain, liver, and prostate tumors that have been transplanted into mice, researchers have found. The treatment, an antibody that blocks a “do not eat” signal displayed on tumor cells, coaxes the immune system to destroy the cells.

A decade ago, biologist Irving Weissman of the Stanford University School of Medicine in Palo Alto, California, discovered that leukemia cells produce higher levels of a protein called CD47 than do healthy cells.



Survivor. When mice with human tumors received anti-CD47, the cancers shrank and disappeared.

CD47 blocks the immune system from destroying blood cells as they circulate. Cancers take advantage of this flag to trick the immune system into ignoring them. Now, Weissman and colleagues have shown that a CD47-blocking antibody can work on a range of tumors.

The scientists exposed tumor cells to macrophages, a type of immune cell. When the CD47 antibody was present, the macrophages destroyed cancer cells from all tumor

types. Human tumors transplanted into the feet of mice and treated with anti-CD47 shrank and did not spread, the team reported online 26 March in the *Proceedings of the National Academy of Sciences*. Weissman's

BY THE NUMBERS

1.875 million joules Record-breaking amount of ultraviolet laser light delivered to the target chamber of the National Ignition Facility at Lawrence Livermore National Laboratory on 15 March.

£100 million New investment pledged 21 March by U.K. Chancellor George Osborne to boost science and engineering research in U.K. universities.

team has received a \$20 million grant from the California Institute for Regenerative Medicine to move the findings to human safety tests. <http://scim.ag/onedrug>

Random Sample

The Science of Judo

Every form of exercise uses a different combination of the body's metabolic systems for energy. It's relatively easy to parse out those combos for cyclical sports like running and cycling using exercise machines in a laboratory. But that's harder to do with more unpredictable sports such as martial arts. So a team of Brazilian researchers have taken the lab into the dojo to study the energy requirements of the Japanese art of judo.

Aerobic metabolism does most of the work during long periods of exercise, such as running a marathon. For shorter, more intense exertion, or when the oxygen runs out, muscles can break down sugar anaerobically. And for very short bursts of energy, muscles can use energy-storing compounds, called phosphagens, in muscular tissues.

To assess how the body gets energy during a more unpredictable sport, Emerson Franchini, a physiologist at the University of São Paulo in Brazil, and his team outfitted judo practitioners with a portable physiology lab: a mask attached to a device worn on the torso that analyzes gases in the martial artist's breath and measures the pulse. The athletes tried different kinds of throws, and also sparred at high intensity.

Metabolically, judo turned out to be a mix of aerobic sports like running and anaerobic sports like weightlifting, the researchers report online this month in the *Journal of Visualized Experiments*. “Normally, judo is thought to be highly lactic” because it requires intense bursts of energy, Franchini says. But the data revealed that phosphagen metabolism was crucial for throwing people, and aerobic metabolism was also higher than expected.

Franchini says the findings should help judo teams train. By knowing their energy expenditures, for example, martial artists can better customize their diet. <http://scim.ag/judomet>



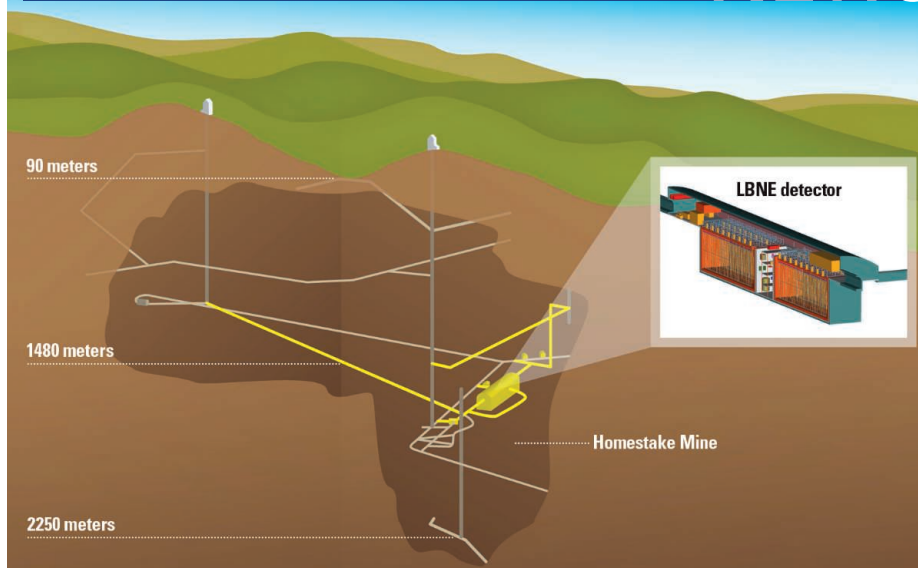
Genome Sequencing Without the Fuss

The potential of using nanotechnology to decipher genetic codes is one step closer to reality. Researchers have demonstrated for the first time that they can continuously read the chemical letters of DNA as it travels through a tiny pore, paving the way for a new kind of sequencing machine that decodes DNA much like an announcer reading a ticker tape. The advance might drop the cost of sequencing a complete human genome below \$1000, which is expected to revolutionize personalized medicine and help usher in a new era of genetic-based diagnostics and medicines.

Nanopore sequencing made headlines in February when Oxford Nanopore Technologies announced it would start selling machines by early next year, but the company released little supporting data. One major problem is that the DNA moves too fast through the pore to be decoded. Now Jens Gundlach, a physicist at the University of Washington, Seattle, has put a protein in the pore that slows the DNA down, and, as reported this week in *Nature Biotechnology*, allows the sequence to be determined. <http://scim.ag/DNAseq>

Science LIVE

Join us on 5 April at 3 p.m. EDT for a live chat with experts on the Comprehensive Nuclear-Test-Ban Treaty. <http://scim.ag/science-live>



PARTICLE PHYSICS

DOE Derails Planning for Major Fermilab Experiment

The already tenuous future of particle physics in the United States now looks even more uncertain. This week, the U.S. Department of Energy (DOE) cited a tight budget in putting the brakes on the development of the flagship project for the next decade at the country's sole particle physics lab, Fermi National Accelerator Laboratory (Fermilab) in Batavia, Illinois. But if the \$1.5 billion Long-Baseline Neutrino Experiment (LBNE) is delayed, then Japanese physicists may be able to do a similar experiment first, researchers say.

LBNE would deploy a gigantic particle detector in the abandoned Homestake gold mine in South Dakota. Filled with 40,000 tonnes of frigid liquid argon, the detector would snare elusive subatomic particles called neutrinos fired through Earth from Fermilab 1300 kilometers away. Physicists would search for an asymmetry between neutrinos and antineutrinos, called CP violation, that might help explain why the universe generated so much matter and so little antimatter.

But LBNE won't fit into DOE's \$792 million particle physics budget, which is unlikely to increase in coming years. "We do not see that we can afford a project as large as that proposed as LBNE," says William Brinkman, director of DOE's Office of Science. In a 19 March letter to Fermilab Director Pier Oddone, Brinkman asked for "the development of an affordable and phased approach [to the experiment] that will enable important science results at each phase."

In addition to the enormous detector in Homestake, LBNE also requires improvements to the mine infrastructure, a new beamline at Fermilab for generating neutrinos, and a smaller detector at Fermilab to compare the hail of neutrinos near the source to the flux that arrives at Homestake. Robert Svoboda, a physicist at the University of California, Davis, and co-spokesperson for the LBNE team, believes that it should be possible to build LBNE piecemeal, with each piece doing science once it is completed. The big detector alone could search for signs that protons decay into other particles, as predicted by so-called grand unified theories, and detect neutrinos from distant supernovas. The smaller detector and the beamline at Fermilab could refine measurements of neutrino parameters and search for so-called sterile neutrinos.

"I'm sure that we will come up with a realistic program that will give us the ability to do this physics," Fermilab's Oddone says. Still, he acknowledges that "any phased approach is going to take longer, be more complicated, and have a lot more reviews."

The stakes for Fermilab are high, physicists say. For 25 years, the lab boasted the world's highest energy atom smasher. It lost that crown in 2010 when the Large Hadron Collider at the European particle physics laboratory, CERN, in Switzerland started taking data. Fermilab leaders have turned from hunting exotic new particles on the "energy frontier" to exploring the "intensity frontier"

Remote sensing. A gigantic underground particle detector in the Black Hills of South Dakota would field neutrinos fired from Illinois.

by generating more intense beams of familiar particles. LBNE would help lay the groundwork for Fermilab's dream machine, an ultra-intense proton accelerator that would amp up the beam to Homestake and generate particles for other experiments.

If LBNE isn't built, Oddone says, "we won't run out of things to do." Others aren't so sure. Marvin Marshak, a physicist at the University of Minnesota, Twin Cities, says he expects DOE will build LBNE because it's essential to the domestic particle physics program. "What's the alternative?" he says. Not doing LBNE "would lead to the decline of Fermilab and the domestic program."

Researchers had been hoping to get LBNE running as early as 2021, but a phased approach to the experiment would push back that start by years. Any major delay could give researchers in Japan the edge in the race to spot CP violation among neutrinos. They have an experiment called T2K, which shoots neutrinos from an accelerator in Tokai to the subterranean Super-Kamiokande particle detector 295 kilometers away. They have plans for an experiment called Hyper-K that would use a much larger detector. Hyper-K would likely cost more than LBNE, Svoboda says.

LBNE aside, Brinkman's letter preserves the hope of scientists that DOE will build an underground lab at Homestake. Plans for such a lab fell apart in December 2010, when the National Science Foundation scrapped an \$875 million proposal for the mine and left the site to DOE. Brinkman says DOE will now pursue the "demonstrator" phase of two smaller experiments in Homestake, the final versions of which would cost a few hundred million dollars. One would try to detect particles of the mysterious dark matter whose gravity binds the galaxy; the other would search for a type of radioactive decay that would prove neutrinos are their own antiparticles. The demos will run in a hall outfitted by the state of South Dakota. "Those are 5-year experiments, so [Brinkman] is saying he's going to run the lab for 5 years," Svoboda says.

But running the lab isn't the same thing as funding LBNE. DOE's plans for the demos could mean that it will pursue, for example, just dark matter experiments and not the vast neutrino detector that physicists want so badly. Brinkman has asked Fermilab physicists to consider that possibility. —ADRIAN CHO

CREDIT: FERMILAB

Biomarker Tests Need Closer Scrutiny, IOM Concludes

A yellow caution flag was urgently waved last week in front of researchers racing to develop complex molecular tests for use in medical care. An Institute of Medicine (IOM) review of flawed research at Duke University in Durham, North Carolina, found many missteps in how scientists and physicians there developed genetic signatures to guide cancer treatment. And the problems aren't limited to Duke: The IOM report concluded that so-called omics tests—diagnostic and prognostic tools based on patterns of nucleic acids, proteins, or other molecules in tissues such as blood—are highly prone to errors; it recommended that any future tests be rigorously validated before their use in clinical trials.

The report also calls on institutions, funders, and journals to take steps to avoid the research management problems it examined at Duke. The Duke case, which led to more than two dozen retracted papers, three cancelled clinical trials, and lawsuits, is “a watershed illustration” of how systems to ensure the integrity of science can fail, the report says. “There are a lot of lessons here that surely apply to other places” doing translational research, says committee chair Gilbert Omenn, a physician-geneticist and computational biologist at the University of Michigan, Ann Arbor, who chaired the IOM committee.

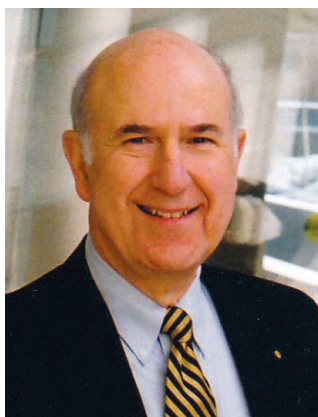
The controversy that spawned the IOM review began when Duke researchers led by physician and cancer researcher Anil Potti and his mentor, cancer geneticist Joseph Nevins, published a series of reports indicating that patterns of gene activity, or gene expression, in tumor cells could be used to predict how an individual would respond to various chemotherapy drugs. The university soon launched three clinical trials in which patients were to receive cancer drugs matched to their gene-expression results.

Two biostatisticians at MD Anderson Cancer Center in Houston, Texas, Keith Baggerly and Kevin Coombes, found apparent errors in the original 2006 *Nature Medicine* paper by the Duke team, as well as in its related publications, and published a critique in September 2009. The National Cancer Institute (NCI), which had wanted to use a Duke gene signature in a new trial but could not verify it, also began to probe. In response to these concerns, Duke stopped the trials for a review of its own but restarted them weeks later.

Then in July 2010, after *The Cancer*

Letter, a Washington, D.C., newsletter, reported that Potti had falsely claimed to be a Rhodes Scholar, more than 30 statisticians and bioinformaticists wrote to NCI Director Harold Varmus asking NCI to investigate. Varmus requested the IOM study. Duke halted the trials a second time and launched a new investigation (*Science*, 6 August 2010, p. 614). Potti left Duke in 2010—a scientific misconduct investigation is ongoing, the university says—and some of the patients in the trials, or family members of deceased patients, have since sued Duke, Potti, and others.

The IOM panel found many potential problems inherent in tests based on finding patterns in large sets of proteins, DNA, RNA,



“There are a lot of lessons here that surely apply to other places.”

—GILBERT S. OMENN,
UNIVERSITY OF
MICHIGAN,
ANN ARBOR

or metabolites. Such molecular tests offer great potential for guiding patient care, but fewer than expected have reached the clinic, the report notes.

One major problem, Omenn and his colleagues say, is “overfitting”: Because the initial studies often look for patterns among hundreds or thousands of biomolecules using a small number of patient samples, it is easy to find false correlations with disease outcomes. The report recommends a set of steps to validate any potential molecular signatures, such as repeating the test on blinded samples from a different institution. Journals and funders should also require that data and models from papers are freely available so that other researchers can check the results.

Those developing a new omics test should also consult with the Food and Drug Administration (FDA) long before they use the test in a clinical trial—which Duke did not do because agency requirements were ambiguous, the report notes. FDA needs to clarify that if such a test will be used to guide

patient treatment, the agency must approve it first, the report says.

With regard to the specific Duke case, the IOM committee also found problems with the university's oversight of the test developed by Potti, declaring that “multiple systems put in place ... to ensure the integrity and rigor of the scientific process failed.” The report notes that financial conflicts of interest—some Duke investigators had patents on technology and ties to companies developing the tests—and deference to a senior professor may have influenced the university to dismiss concerns about the papers. (A Duke official attributed failure to “missed signals,” the report says.) To avoid these problems, the IOM panel says institutions need to strengthen their oversight of conflicts of interest and processes for responding to questions about published research.

In a statement, Duke said it expects to incorporate the recommendations into “ongoing efforts to strengthen the rigor of our research enterprise. We have learned from our situation.”

Although the Duke case involved a total of 162 co-authors across 40 papers, two-thirds of which are being partially or completely retracted, “not a single one of those investigators had raised a peep about the transparent flaws in these original papers,” Omenn notes. What happened reflects “a rush” to move genomics-based tests into the clinic and commercialize them, he says. As other institutions respond to a push to move basic research more quickly into the clinic, “this is a big wake-up call,” Omenn says. “It's got to be done right.”

MD Anderson's Baggerly says his initial read of the report is “quite positive.” If the recommendations had all been in place at Duke, “I suspect many of the problems encountered might have been short-circuited.”

NCI biostatistician Lisa McShane, who spent months trying to validate the Duke results, says the institute now plans to require any large clinical trial groups it funds that want to use an omics test to follow a checklist similar to that recommended in the IOM report. “Our hope is that this report will heighten everyone's awareness,” McShane says. “We now recognize in a painful way what the consequences can be [when flawed omics research goes undetected], and we need to fix it.”

—JOCELYN KAISER



AGRICULTURE

Field Research on Bees Raises Concern About Low-Dose Pesticides

Five years ago, bees made headlines when a mysterious condition called colony collapse disorder decimated honey bee colonies in parts of the United States (*Science*, 18 May 2007, p. 970). Now bees are poised to be in the news again, this time because of evidence that systemic insecticides, a common way to protect crops, indirectly harm these important pollinators. Two field studies reported online this week in *Science* document problems (<http://scim.ag/MHenry>, <http://scim.ag/Whitehorn>). In bumble bees, exposure to one such chemical leads to a dramatic loss of queens and could help explain the insects' decline. In honey bees, another insecticide interferes with the foragers' ability to find their way back to the hive.

Researchers say these findings are cause for concern and will increase pressure to improve pesticide testing and regulation. "It's going to cause an absolute firestorm," predicts James Cresswell, an ecotoxicologist at the University of Exeter in the United Kingdom, who was not involved in the research. Bayer CropScience, the main producer of systemic pesticides, maintains that its products are not a culprit in honey bee declines, and many independent experts aren't convinced by the evidence against pesticides, adding that pathogens and parasites are the main problem.

In the United States alone, 59 million hectares of crops are protected by systemic pesticides. Seeds are treated with these neurotoxins before planting, and the poison suffuses the tissues, pollen, and nectar. Typical levels of imidacloprid, the most common of a family of widely used pesticides called neonicotinoids, are not lethal to bees, according to a meta-analysis by Cresswell published in

Ecotoxicology last year. But there is growing evidence from laboratory experiments that neonicotinoids can harm memory and navigation in bees. However, realistic field tests were lacking.

David Goulson, a bumble bee biologist at the University of Stirling in the United Kingdom, and colleagues have now studied the bumble bee *Bombus terrestris* under seminatural conditions. Mimicking the bees' exposure to imidacloprid in canola, they fed bumble bees a controlled diet in the laboratory. Twenty-five colonies received pollen treated with six parts per billion of the pesticide. Another 25 colonies got double that dose, and 25 more served as a control. After 2 weeks, the blooming period for canola, the researchers placed the colonies in a field for 6 weeks to forage on gardens, wildflowers, and a variety of crops.

At the end of the experiment, the hives with the bees that had eaten the imidacloprid in the lab weighed 8% to 12% less than the 25 untreated hives—an indication that the bees had gathered less food and produced fewer workers. The most important difference was the number of queens produced; the control hives averaged 13 queens, compared with 2 and 1.4 in the treated hives. "That is quite dramatic," Dennis vanEngelsdorp of the University of Maryland, College Park, says. It suggests that pesticides may be contributing to the decline of bumble bees, which are already suffering from habitat loss.

Tjeerd Blacquière of Plant Research International in Wageningen, the Netherlands, who was not involved in the study, says Goulson's results are probably a worst case scenario because the affected bumble bees

Royal pain. Bumble bee hives produce fewer queens (top) when exposed to pesticides.

were forced to consume only one kind of pollen—pesticide tainted—in the lab, whereas they would probably have a choice in the wild, thus lowering the dose.

Why the hives produced fewer queens isn't clear, but Goulson says that if imidacloprid hinders the navigation ability of the foragers, they might not have gathered enough food for the queen to reproduce more queens.

Foraging problems are exactly what Axel Decourtye of the Association for Technical Coordination in Agriculture in Avignon, France, and his colleagues found in a field study of honey bees. Decourtye's team glued tiny radio-frequency tags to the backs of 653 honey bees. Up to 43.2% of the bees given a sublethal dose of thiamethoxam didn't return to the hive, depending on how far away the bees were released and how unfamiliar the terrain, compared with 16.9% of untreated bees. "We were quite surprised by the magnitude of the effect," co-author Mickaël Henry of the French National Institute for Agricultural Research in Avignon says.

The team plugged the mortality rates into a model of honey bee population dynamics and found that many colonies would dwindle. Jeffrey Pettis of the U.S. Department of Agriculture in Beltsville, Maryland, doubts that the mortality rates would cause colony collapse disorder or other loss of hives, but says that he is a co-author of a study nearing publication that will strengthen the case that neonicotinoids can harm hives. Other unpublished work shows an impact on native, solitary bees, he says.

David Fischer, an ecotoxicologist at Bayer CropScience in Research Triangle Park, North Carolina, says both studies used doses that were higher than what he thinks is present in crops. Nailing down the actual levels should be a priority, Cresswell says.

The findings reinforce the recommendations of a major report, issued last September under the auspices of the Society of Environmental Toxicology and Chemistry and written by scientists from universities, industry, and regulatory agencies. They called for more sensitive tests, such as feeding pesticides to larvae and immature bees, and including bumble bees and native bees as well.

Regulatory agencies are starting to move, if slowly. The European Food Safety Authority is considering new guidelines for risk assessments of pesticides for bees. For its part, the U.S. Environmental Protection Agency will convene a scientific advisory panel to address similar questions in the fall. —ERIK STOKSTAD



OCEAN SCIENCE

U.S. Budget Cuts Threaten to Sink Undersea Research Fleet

A U.S. government program that provides ocean researchers with access to submersibles and a sea-floor observatory may be doomed to Davy Jones's locker by fiscal belt-tightening—unless Congress steps in to save it. Last week, researchers began to plead their case, asking lawmakers to reject an Obama Administration plan to eliminate the \$4 million National Undersea Research Program (NURP), which is run by the National Oceanic and Atmospheric Administration (NOAA). NURP has survived similar near-death experiences in the past, but researchers fear this year's battle could mark the end of the 32-year-old program.

NURP is “a vital and irreplaceable” part of NOAA's grants program for university researchers and should be restored, marine biologist Shirley Pomponi of Florida Atlantic University's Harbor Branch Oceanographic Institute in Fort Pierce told a subpanel of the House Committee on Appropriations on 22 March.

NOAA created NURP in 1980, on the advice of the National Research Council, to forge partnerships between the agency and academia and take advantage of advances in deep-diving capabilities, says Andrew Shepard, a benthic ecologist at the University of South Florida (USF), Tampa, and executive director of the Gulf of Mexico University Research Collaborative. The program has funded more than 500 projects since 2000, including some 1000 submersible dives and 53,000 SCUBA dives, according to government statistics. NURP efforts have included studying the explosive formation

of Hawaii's newest island, Lo'ihī volcano, as well as collecting decades' worth of data on shallow-water coral reefs.

NURP's budget has declined sharply over the years, from a high of \$18 million to about \$10 million in 2006. But the program is still one of the top three sources of funding for deep-sea science in the United States, says Lisa Levin, a deep-sea biologist at the Scripps Institution of Oceanography in San Diego, California. (The other two are NOAA's Office of Exploration and the National Science Foundation.) “It was never a large amount of money, but I've taken a lot of students to sea through NURP funding,” she says.

In addition to supporting scientific brains, NURP also funds academia's undersea brawn, including two out of the three human-operated research submersibles operated by U.S. universities. (The exception is the celebrated Alvin submarine, run by Woods Hole Oceanographic Institution in Massachusetts.) NURP-supported assets also include remotely operated vehicles (ROVs) that are tethered to ships and robotic autonomous underwater vehicles. The program also funds investigators to work at NOAA's Aquarius Reef Base, an underwater laboratory that sits in 18 meters of water 5.5 kilometers off the coast of Key Largo, Florida.

NURP's elimination could be especially problematic for one of its West Coast regional centers, the Hawaii Undersea Research Laboratory (HURL) at the University of Hawaii, Honolulu. NURP has traditionally covered the costs of operating HURL's two human-

Threatened habitat. NOAA's Aquarius Reef Base off Florida could host its last scientific team in July if Congress doesn't reinstate funds for NURP, which supports the underwater laboratory.

piloted submersibles, Pisces IV and V, and officials were counting on the program to also support a recently purchased \$2 million ROV. If NURP is shut down, “we'll lose two of the three submersibles,” predicts Bruce Robison, a deep-sea ecologist with the Monterey Bay Aquarium Research Institute in Moss Landing, California. Losing NOAA funding for the subs will make it harder for U.S. researchers to get a firsthand, three-dimensional view of the deep sea, he says. In contrast, using cameras mounted on ROVs to observe marine environments “is like looking with one eye through a pipe.”

Losing the expertise to run these vehicles is equally worrisome, says HURL Director John Wiltshire, who specializes in offshore energy and mineral resources. “It takes us 5 years to train a good sub pilot, and these people will be snapped up by the oil industry in a month,” he says.

It is less clear what will happen to NURP-funded assets if the program is defunded, although NOAA officials say they plan to get rid of Aquarius Reef Base, the Pisces V submersible, and other vehicles by the end of fiscal year 2013. But NURP-supported scientists should be able to find replacement funds elsewhere, NOAA officials say. “NOAA had to curtail even some important programs, particularly where other avenues of funding might be pursued,” wrote NOAA spokesperson Scott Smullen in an e-mail. “NURP is one of those programs.”

This isn't the first time the White House has eliminated NURP funding from its annual budget request. But in the past, political champions in Congress, including senators from coastal states, have successfully argued to reinstate the funds. This year could be different, given that some of those members of Congress have retired or died, according to NURP advocates. “We just don't have that political clout anymore,” says USF's Shepard.

In some ways, NURP is a victim of its own success, Shepard says. NOAA is now using technologies that NURP helped develop, and the agency has benefited from the specialized workers NURP has helped train. “It's like someone looked at [NURP] and said: ‘Congratulations, we don't need you anymore.’” NURP's fate will be decided later this year, as Congress shapes the 2013 budget.

—JANE J. LEE

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NONPROLIFERATION

North Korea Conundrum Overshadows Nuclear Summit

SEOUL—On a visit to the Yongbyon nuclear complex in November 2010, Siegfried Hecker, a plutonium expert at Stanford University, wasn't expecting a big surprise. It was his seventh time there at North Korea's invitation to bear witness to its nuclear prowess. When Hecker visited in January 2004, his hosts handed him a glass jar with plutonium metal harvested from the spent fuel of a Yongbyon nuclear reactor. This confirmed that North Korea had the raw material for a bomb before the isolated nation conducted nuclear tests in 2006 and 2009. No subsequent visit rivaled that experience—until 2010, when Yongbyon managers ushered Hecker into a building as long as a football field.

From a second-floor observation platform, Hecker gazed down on a technological marvel: around 2000 neatly plumbed uranium enrichment centrifuges. The “astonishingly modern” computer controls and clean-room feel were a world apart from the other ramshackle Soviet-era facilities at Yongbyon. “It was nothing like I'd seen before in North Korea,” Hecker says.

Revelations about North Korea's nuclear program and its latest saber rattling have lent urgency to an international effort to keep nuclear materials out of the hands of terrorists or rogue states. At the Nuclear Security Summit here earlier this week, top officials from 53 nations lauded an initiative begun 2 years ago to secure highly enriched uranium and other fissile material. But the talks were overshadowed by Iran's continued defiance over its uranium enrichment program and North Korea's plans to launch a rocket that the United States and others assert would be a long-range ballistic missile test in violation of U.N. sanctions.

From the south side of the demilitarized zone, the menace looms large. U.S. President Barack Obama and other leaders held bilateral talks here on the summit's sidelines about how to curb the North's nuclear ambitions. Obama declared that the rocket launch would jeopardize a 29 February agreement with the United States in which North Korea promised to freeze activities at Yongbyon and abstain from nuclear and ballistic missile tests in exchange for 240,000 tons of food aid. North Korea has denied that it will conduct a missile test; it says the launch is to put an Earth-

observation satellite in orbit.

Veteran analysts are mystified over North Korea's decision. “It's an unambiguous, deliberate renege,” Leon Sigal, a security expert at the Social Science Research Council in New York City, said at a meeting here last week to mark the 40th anniversary of Kyungnam University's Institute for Far Eastern Studies. After months of efforts to restart stalled denuclearization talks, he predicts, “a rocket launch would be confidence destroying. Fruitful dialogue will come to an end.”



Perceived threats. Siegfried Hecker examines equipment at the now-defunct Yongbyon nuclear fuel facility; a controversial 2009 North Korea rocket launch (left).



Insights into North Korea's nuclear program and the intentions of its new leader, Kim Jong Un, are critical to the global effort to safeguard nuclear materials. One major worry is North Korea's track record in exporting nuclear secrets. It allegedly helped Syria build a clandestine nuclear reactor destroyed by an Israeli air strike in 2007; earlier that decade, the International Atomic Energy Agency concluded, it “very likely” supplied Libya with tons of uranium hexafluoride gas, the feedstock for enriching uranium in centrifuges.

In 2002, U.S. diplomats confronted North Korean officials over intelligence reports that Korean entities were acquiring aluminum alloys, frequency converters, and other equipment necessary to build high-precision centrifuges. North Korea strenuously denied the existence of an enrichment program. Nuclear experts were unsure of the evidence until Hecker's tour in 2010, which confirmed

the intelligence reports. Hecker suspects that North Korea began experimenting with enrichment as early as the 1970s, around the time its nuclear scientists “went solo” after early Soviet help. In the 1980s, they built a gas graphite reactor capable of generating a bomb's worth of plutonium a year and a reprocessing facility to extract the material from the spent fuel. Western nations, Hecker says, have underestimated North Korea's young nuclear scientists, who he was told run Yongbyon's uranium enrichment workshop.

Hecker's minders insisted in 2010 that the facility is meant to produce low-enriched uranium for a light water reactor under construction at Yongbyon. “It's a great cover story,” Hecker says. “To run a modern light water reactor, you need 60,000 centrifuges. For a nuclear weapons program, 2000 or 3000 can get you a bomb or two per year.” But why would North Korea pursue uranium if it already has enough plutonium for several bombs? “They may think they can miniaturize [a uranium warhead] quicker than plutonium,” Hecker says. If they succeed at that task, they would need a delivery vehicle. “For a deterrent to be effective,” he notes, “you have to be able to threaten someone.”

That's why tensions are high over next month's rocket launch. Another worry is a mobile medium-range missile launcher that North Korea unveiled at a military parade in October 2010. The movable “Musudan” launcher could be hidden easily, says Hecker, who notes that the system hasn't been tested and, therefore, is not a threat “for the foreseeable future.” Some experts wonder if it ever will be. Taik-young Hamm, a political scientist here at the University of North Korean Studies, doubts whether North Korea has the finances or know-how to accrue a “sizable” nuclear arsenal.

Others insist that North Korea has the wherewithal to expand its arsenal through uranium enrichment. The biggest unknown may be what it will take to induce the North to relinquish its nukes—if it has a price. “Signs indicate that North Korean leaders are becoming less willing to give up nuclear weapons,” says Jae Kyu Park, president of Kyungnam University in Changwon. Sigal agrees. “Denuclearization is not for sale,” he says. But patience could pay off, says Hecker, who sees North Korea as “an island of instability” in a sea of stability and prosperity: “We have to convince them that their nuclear weapons are a liability.”

—RICHARD STONE



Psychology's Bold Initiative

In an unusual attempt at scientific self-examination, psychology researchers are scrutinizing their field's reproducibility

PICK UP THE JANUARY 2008 ISSUE OF *Psychological Science*, turn to page 49, and you'll find a study showing that people are more likely to cheat on a simple laboratory task if they have just read an essay arguing that free will is an illusion. It was a striking study that drew widespread attention both from psychologists and from many media outlets. But should you believe the result?

There's no reason to think that the study, conducted by psychologists Kathleen Vohs of the University of Minnesota Carlson School of Management in Minneapolis, and Jonathan Schooler, who is now at the University of California, Santa Barbara (UCSB), is incorrect. Yet according to many psychologists, their field has a credibility problem at the moment, and it affects thousands of studies like this one.

Part of the angst stems from recent high-profile cases of scientific misconduct, most dramatically the extensive fraud perpetrated by Dutch social psychologist Diederik Stapel (*Science*, 4 November 2011, p. 579), that have cast a harsh light on psychological science. Yet there is no evidence that psychology is more prone to fraud than any other field of science. The greater concern arises from several recent studies that have broadly critiqued psychological research practices, highlighting lax data collection, analysis, and reporting, and decrying a scientific culture that too heavily

favours new and counterintuitive ideas over the confirmation of existing results. Some psychology researchers argue that this has led to too many findings that are striking for their novelty and published in respected journals—but are nonetheless false.

As a step toward testing that disturbing idea, one project begun this year offers an online site (PsychFileDrawer.org) where psychologists can quickly and easily post, in brief form, the results of replications of experiments—whether they succeed or fail. University of California, San Diego, psychologist Hal Pashler, one of the project's developers, says the goal is to counteract the “file



Double trouble? Brian Nosek leads a large-scale effort to replicate recent psychology studies

drawer problem” that plagues all of science, including psychology; researchers usually just file away straightforward replication studies because most journals decline to publish such work.

In an even more daring effort, a group of more than 50 academic psychologists, which calls itself the Open Science Collaboration (OSC), has begun an unprecedented, large-scale project to systematically replicate psychological experiments recently published in leading journals. “We’re wringing our hands worrying about whether reproducibility is a problem or not,” says psychologist Brian Nosek of the University of Virginia in Charlottesville, who is coordinating the effort. “If there is a problem, we’re going to find out, and then we’ll figure out how to fix it.”

Robert Kail, a Purdue University developmental psychologist and editor of *Psychological Science*—one of the three journals whose papers the OSC is attempting to replicate—is optimistic that a high percentage of published findings will be replicated. Nonetheless, he views the field’s recent attention to the issue of false positives as healthy. “There has been a lot of speculation about the extent to which it’s a problem,” he says. “But nobody has actually set it up as an empirical project. It’s a great thing for somebody to actually do that.”

Schooler, who is not directly involved with the project but whose free will study

will be replicated, considers the OSC replication study a “bold initiative.” Yet he’s concerned that if the project confirms few studies, it could unfairly indict psychology. “I think one would want to see a similar effort done in another area before one concluded that low replication rates are unique to psychology,” he says. “It would really be a shame if a field that was engaging in a careful attempt at evaluating itself were somehow punished for that. It would discourage other fields from doing the same.”

Indeed, the prospect of exposing psychology’s foibles has upset some scientists. “I had a senior person in the field ask me not to do it, because psychology is under threat and this could make us look bad,” Nosek says. “I was stunned.” The point of the project, he says, is not to single out individual studies or disparage psychology. “We’re doing this because we love science. The goal is to align the values that science embodies—transparency, sharing, self-critique, reproducibility—with its practices.”

Am I doing something wrong?

Reproducibility is supposedly a basic tenet of science, but a number of fields have raised concerns that modern publishing pressures inhibit replication of experiments. In a well-known 2005 *PLoS Medicine* essay, epidemiologist John Ioannidis, now at Stanford University in Palo Alto, California, argued that in biomedicine, many if not most published research claims are false. He outlined a number of factors—including small sample sizes, small effect sizes, and “flexibility” in the research process—that contribute to a high rate of false positives. One reason those false positives aren’t caught is because of a lack of emphasis on replication studies, which is “standard across fields of science,” says Columbia University statistician Victoria Stodden, who studies reproducibility and openness in computational science.

Nosek first became interested in the problem of replication in psychology several years ago, after he started having persistent problems confirming others’ results—something his lab routinely does before extending research to address new questions. Believing himself to be a careful methodologist, he wondered whether something was wrong with the studies he was attempting to reproduce.

For example, many social psychological studies have asked participants to unscramble sentences containing words that connote a particular concept that the researchers aim

to “prime” in the participants’ minds—say, impulsivity, or anger, or happiness; the idea is to test the effects of those mental constructs on a subsequent task. The scrambled-sentence task has been used in many published studies, but Nosek’s group has rarely been able to get it to work, and he suspects that the effect may be much more limited than the published literature would suggest.

The idea of systematically testing the reproducibility of psychological science percolated in Nosek’s mind until late last year, when revelations of Stapel’s scientific misconduct brought the issue to a boil. Stapel, whose studies were widely cited and had drawn frequent media attention, admitted last fall to fabricating data on more than 30 studies dating back to the mid-1990s. One of those high-profile studies, published in *Science* and now retracted, concluded that chaotic physical surroundings promote stereotyping and discrimination.

News of Stapel’s fraud led many psychologists to question whether the field possesses sufficient checks and balances to prevent such willful deception. It also stirred some researchers’ nascent worries that—rare acts of

their materials, yet old enough for the OSC to analyze questions such as whether a study’s reproducibility correlates with how often it has been cited subsequently. Many psychology articles include more than one study to support their conclusions, but the OSC decided in advance to select only the final study from each article; if that study was not feasible to replicate, they worked backward until an eligible study was identified.

After identifying eligible experiments, individual OSC members began choosing the ones each would repeat and contacting the original authors to obtain materials and fill in methodological details. So far, the response from original authors has been overwhelmingly positive—only two have declined to provide materials needed for replication.

More than 30 replication studies are now under way, and the group aims to conduct at least 50. (New researchers can join the collaboration anytime.) The results OSC members will seek to reproduce vary widely, from a study of how photos of unsmiling black men automatically activate attention in some people’s minds, to a study that examined how the timing in which perceptual stimuli are presented affects short-term memory.

The replication studies are being funded by the OSC researchers who select them; however, in keeping with the samples used in the original studies, most will be cheap because they’ll use undergraduates who participate for course credit—although using study populations that are mostly limited to young, white college students from Western industrialized cultures has its own drawbacks (*Science*, 25 June 2010, p. 1627).

Repeat after me

While reproducibility is often held up as the “gold standard” in science, Nosek argues that “direct replications,” in which researchers follow an original experiment’s procedure as closely as possible, are rare. “There is no incentive for replication—it’s all about the new idea,” he says.

Not all psychologists agree. Social psychologist Norbert Schwarz of the University of Michigan, Ann Arbor, says that although the OSC project is valuable, concern about the field’s robustness may be overblown. Direct replications may be rare, but Schwarz points out that conceptual replications—in

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Podcast interview
(http://scim.ag/pod_6076) with author
Siri Carpenter.

“IT WILL BE A FIRST ESTIMATE OF THE REPRODUCIBILITY OF FINDINGS THAT ARE IN IMPORTANT JOURNALS IN PSYCHOLOGY.”

—BRIAN NOSEK, UNIVERSITY OF VIRGINIA

fraud aside—commonly accepted practices in psychology research might produce an unacceptably high number of false positive results. “It just became obvious that it was time to do some science to figure it out,” Nosek says.

In November 2011, Nosek approached a few departmental colleagues to propose a collaborative effort to study the field’s reproducibility. The effort soon expanded to include researchers from universities in the United States, Canada, and Europe.

This winter, OSC members began identifying studies to include in their replication sample. They chose three high-impact psychology journals—*Psychological Science*, the *Journal of Personality and Social Psychology*, and the *Journal of Experimental Psychology: Learning, Memory, and Cognition*—and began logging key details of the first 30 articles published in each journal in 2008. They reasoned that articles published during this time frame are recent enough that most original authors can find and share

which researchers tweak a study's materials or procedure to test a hypothesis in a different way—make up the bulk of psychology's literature and “are highly valued because they look at the stability of a phenomenon across different content domains.”

Nosek agrees that conceptual replications are important and common, and temper worries that journals are littered with false findings. But conceptual replication assumes that the replication addresses the same phenomenon as the original demonstration, which may not always be the case, he says.

Direct replications are important, he and others say, because psychology, like other disciplines, has practices that may lead to too many false positives. In the November 2011 issue of *Psychological Science*, University of Pennsylvania psychologist Joseph Simmons and colleagues showed, through computer simulations and actual experiments, that “flexibility” in research decisions such as how many research subjects to include in

There is currently nothing to prevent such “motivated reasoning,” as psychologists call it, from contaminating the scientific literature.

Looking ahead

Nosek isn't just depending on OSC's look at past studies to improve psychology research practices. He's also looking forward and has developed, with his graduate student Jeffrey Spies, an online database (openscience-framework.org) where psychological scientists can easily organize and—if they choose—register study materials, hypotheses, planned analyses, and data. Nosek hopes that in addition to aiding laboratory workflow and providing an outlet for results that might not otherwise see the light of day, the registry will increase researchers' sense of accountability both to their community and to themselves. Looking forward to using the registry for his own research, Nosek says he sees it as “a way of guarding the truth against my own motivated reasoning.”

those that were single-study papers, that might hint that “bite size” reports are problematic, as some critics have suggested.

One limitation in interpreting the OSC's results stems from the fact that the group is not tackling a representative sample of all psychology research. Studies that used statistical analyses that cannot yield clear evidence of replication have been excluded, as have studies that would be infeasible to replicate because they require specialized materials, instrumentation, or participant populations. Furthermore, most studies included in the sample are drawn from cognitive and social psychology; other subfields, such as developmental and clinical psychology, neuroscience, and animal behavior, are not included.

Stanford University social psychologist Nalini Ambady says several junior colleagues have told her they're worried about this disproportionate focus because if a high percentage of OSC studies fail to be replicated, many people may conclude that it is social psychology alone that is problematic. She sympathizes with that argument. “I think if you want to do it, then you should do a fair, representative sampling,” Ambady says. “Don't just focus on the social psychological studies that are the low-hanging fruit because they are generally cheapest and easiest to conduct.”

The study's heavy focus on social and cognitive psychology is a reflection of the researchers who have gotten involved so far, Nosek responds: “If there are people in other subfields who want to join the project, we will be delighted to broaden it.” The OSC won't produce a “definitive study,” he stresses. “It will be a first estimate of the reproducibility of findings that are in important journals in psychology.”

Columbia's Stodden agrees that psychology's efforts to address the issue shouldn't be cause for criticism. Psychologists' scrutiny “is very admirable,” she says. “I think other fields could benefit from that kind of self-reflection.”

Cognitive psychologist Rebecca Saxe of the Massachusetts Institute of Technology in Cambridge, who is participating in the collaboration, is also optimistic. The project, she says, “has the potential to be spun as negative and nihilist. But to me, it's the opposite. Science is about discovering true things, and when you do find something that's true enough that others are able to replicate it, that's just thrilling.”

—SIRI CARPENTER

Siri Carpenter, a freelance writer based in Madison, Wisconsin, worked 12 years ago in a lab with Brian Nosek, the organizer of the Open Science Collaboration.

“IT WOULD REALLY BE A SHAME IF A FIELD THAT WAS ENGAGING IN A CAREFUL ATTEMPT AT EVALUATING ITSELF WERE SOMEHOW PUNISHED FOR THAT. IT WOULD DISCOURAGE OTHER FIELDS FROM DOING THE SAME.”

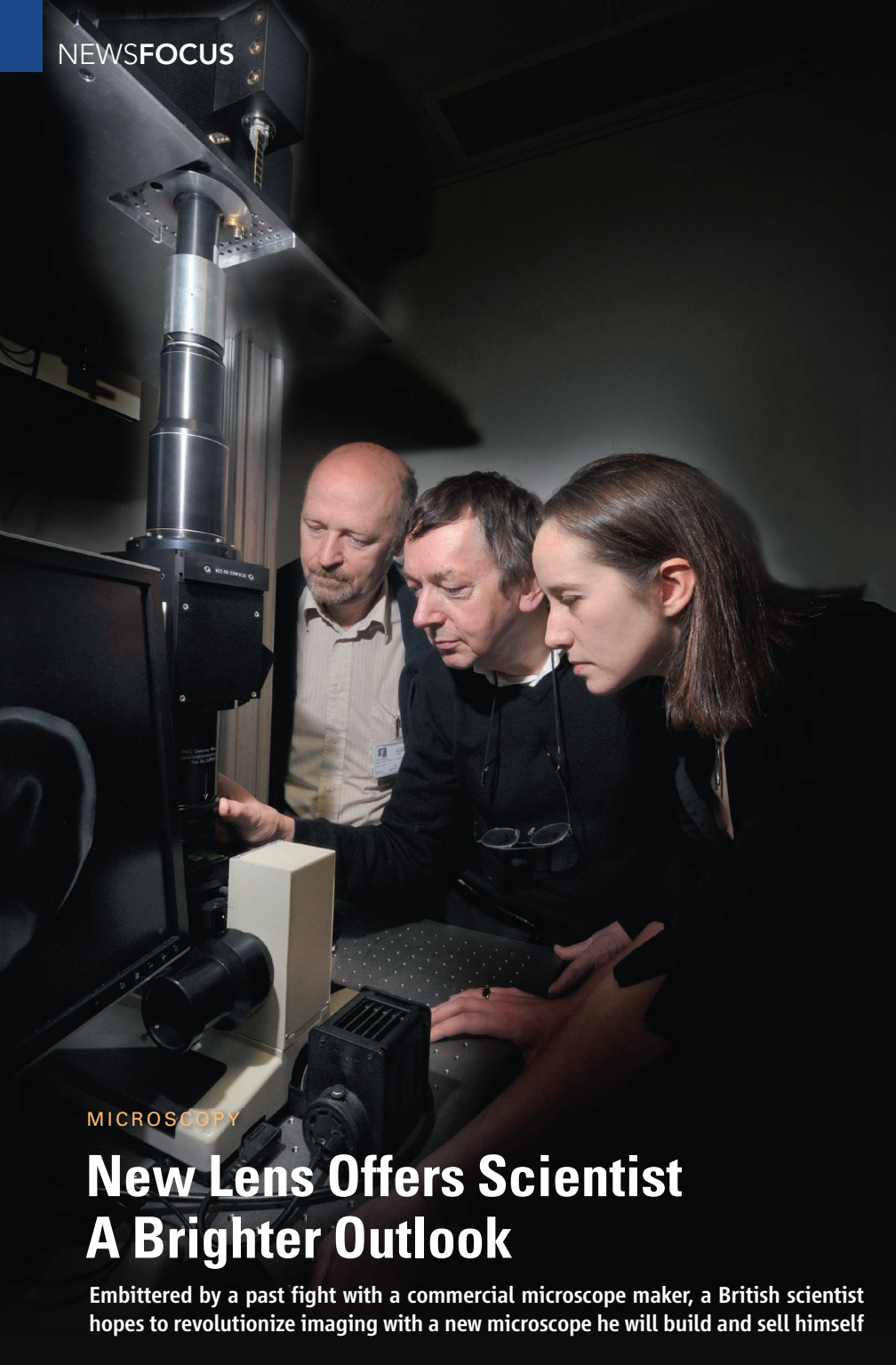
—JONATHAN SCHOOLER, UNIVERSITY OF CALIFORNIA, SANTA BARBARA

a study, how many outcomes to measure, or whether to break down analyses according to participants' gender can more than double the chances of getting a false positive. When several such “researcher degrees of freedom” are in play, as is commonly the case, a study is more likely than not to mistakenly yield a statistically significant effect.

Nosek believes the prevalence of research practices that lead to unintended bias is rooted in the fact that negative results are virtually unpublishable—a state of affairs that has grown more extreme in recent years, especially in psychology. “It is important to my career that I do studies that are publishable,” he says. One way to do that is to capitalize on the kinds of practices that Simmons and colleagues identified. Another is to run many studies with small numbers of subjects and publish the ones that “work.” A third and especially insidious problem, Nosek says, is that researchers can easily fool themselves into believing that chance positive results are actually what they had hypothesized all along—then publish such findings as though they were confirmatory tests of existing hypotheses.

As for the OSC's replication project, data collection on the currently chosen studies should be completed by the end of this year, and Nosek hopes the results will be published not long after. One challenge for the OSC researchers will be in setting criteria for what constitutes a successful confirmation, given that a replication can take varying forms, from a result that has the same statistical significance as the original finding to a pattern of results that is merely in the same direction as the original. That makes it difficult to say what percentage of failed replications should be considered problematic. And it's one reason that Nosek believes the most interesting results of the study may lie not in the raw rate of reproducibility but in what factors predict a study's reproducibility.

For example, if the studies that replicate best are also the most widely cited, that would suggest that despite biases in publishing practices, scientists can separate the wheat from the chaff. If studies that were published along with several others in a multistudy paper—a formulation that often involves multiple confirmations of the same basic effect, at least conceptually—replicate more often than



MICROSCOPY

New Lens Offers Scientist A Brighter Outlook

Embittered by a past fight with a commercial microscope maker, a British scientist hopes to revolutionize imaging with a new microscope he will build and sell himself

Against a wall behind his lab bench, Brad Amos keeps a framed pencil sketch of a gnat larva. He drew the picture as a schoolboy in 1961, viewing the 2-millimeter-long critter through a small brass microscope made by the German optics company Carl Zeiss. “That was my first microscope,” says Amos, an emeritus research group leader at the U.K. Medical Research Council’s Laboratory of Molecular Biology in Cambridge.

It was far from his last. Amos went on to become one of the designers of the laser scan-

ning confocal microscope, famous for producing crisp images of cells by cutting out the blurry flare that came from using standard fluorescence microscopes, a staple of modern cell biology. The laser confocal hit the market in 1986, and Amos guesses there must be between 5000 and 10,000 such devices in use today. He can’t be sure, he says, because he lost control over the invention in the 1990s after a bitter patent dispute with Carl Zeiss. (Other microscope manufacturers, including Leica and Olympus, make confocals as well.)

Better vision. Amos—flanked here by John Dempster and Gail McConnell—has been working on the Mesolens since 2000.

Now, at age 66, Amos has his eyes set on another revolutionary microscope, which he has worked on since 2000. It utilizes an invention he calls the “Mesolens,” a lens capable of capturing a similar level of detail as a confocal alone, but on a far larger scale. Where existing microscopes have lenses up to 3 centimeters long, the Mesolens is a massive half a meter, which means it can capture a specimen as big as a mouse embryo in a single shot.

The Mesolens pushes other boundaries as well. One of the novelties of a confocal microscope is that it can view up to 0.22 micrometers below the surface of a specimen, provided it is translucent enough. When used inside a tailor-made confocal microscope, the Mesolens plumbs depths of up to 3 millimeters. The final effect is a 3D picture that allows one to zoom in as far as the level of the cell. (The name is derived from the Greek *meso*, or middle, because it’s somewhere between a conventional microscope lens and a close-up photographic lens.)

The microscope could save biologists the effort of patching together hundreds of small high-resolution images if they want a larger one. “It would probably take 1 day to image a mouse embryo with a standard confocal microscope using a stitching and tiling method. With the Mesolens, it takes only an hour,” says Amos’s colleague Gail McConnell, a biophotonics professor at the University of Strathclyde in the United Kingdom.

Amos has only a prototype for now, assembled in Strathclyde with the help of McConnell and John Dempster, an imaging software designer. But the device has already created ripples of excitement among scientists. Inquiries have been coming in ever since Amos revealed the first confocal pictures from the Mesolens at the Royal Society in London in early February.

“I want to be his first customer,” says Scott Fraser, a professor of biology and an imaging expert at the California Institute of Technology in Pasadena, who is confident that the Mesolens will break new ground in microscopy. The microscope has obvious biomedical applications, Amos says, such as imaging cancerous tissues, but he has also been contacted by a geologist interested in using the Mesolens to study pores in rocks.

Lucky encounter

Few people know how tough the journey has been for Amos. In his early career as a biologist, he discovered the calcium binding pro-

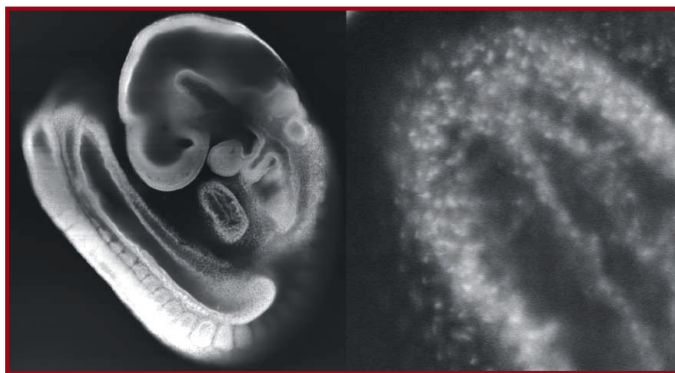
tein, spasmin. But a professional crisis in 1980, following the death of a patron of his work, left him depressed and purposeless. A lucky encounter with John White, a developmental biologist with a background in physics and electronics who had already started work on the confocal microscope, prompted Amos's first foray into microscopy. "I saw John's prototype and I immediately saw how I could contribute to that," he says, adding that he always "liked making things with my hands."

But then came another setback. After the confocal microscope went into production, he was hit with years of litigation by Carl Zeiss, which claimed that the patent on his design was invalid because aspects of it had been developed earlier. By the end of the 1990s, Amos had lost the case—and his invention. "It was a mess," he says.

But he did not lose heart. Instead, Amos began exploring the possibility of a microscope with an enormous lens. The problem was, the bigger it was, "the higher the angle through which the glass has to bend the light, and the greater the likelihood of aberrations," he says.

Lengthy lens. About half a meter long and 8 centimeters wide, the Mesolens produces images that are too big to be seen by the human eye through a viewfinder. So a camera is placed to the left to capture the pictures. Among the 14 lens elements are tilted plates in the center, which reflect light through the lenses toward the specimen on the right and correct for optical errors.

Amos ended up working with Esmond Reid, a British freelance optical designer with expertise in giant telescopes. Together they tinkered with different lenses, trying to find one that would maximize the size of the image without sacrificing too much resolution. "I thought it might be possible, but I was no more than 50% confident," Amos says. "The business of lens design is arcane. There is so much secret knowledge that isn't available to researchers like me but held by



A thousand words. A confocal Mesolens image of a 10-day-old mouse embryo, approximately 3 millimeters across (left), and a 350-micrometer section within the same image, showing the developing heart with individual cells (right).

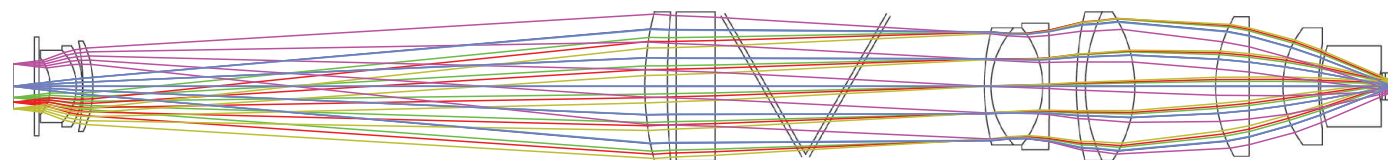
Amos intends to do neither. His battle with Carl Zeiss has soured his view of industry. "It was a rude awakening into the nature of companies, and I'm afraid I've never changed my opinion. It's

a nasty business," he says. In any case, no optics companies have approached him with an interest in producing the Mesolens.

Instead, Amos plans to develop and manufacture the device himself. Even without patents, he is confident that no company has the research and development expertise to make a copy. He has started a company with Reid and has already made substantial investments from his own savings, even carrying out some of the technical work from a workshop in the garden behind his home. "I have two lathes, two milling machines. I can do precision metalwork from there," he says. "I will grow the business by the bootstraps."

Amos intends to do neither. His battle with Carl Zeiss has soured his view of industry. "It was a rude awakening into the nature of companies, and I'm afraid I've never changed my opinion. It's

Amos intends to do neither. His battle with Carl Zeiss has soured his view of industry. "It was a rude awakening into the nature of companies, and I'm afraid I've never changed my opinion. It's



commercial manufacturers." It took the pair a year to hit an acceptable balance. Another technical barrier, which emerged later, was making sure the laser beam that scanned specimens didn't produce a jittery picture. Modern lasers are reliably stable, but the mirrors inside the microscope that reflect these beams are less so. "One of the big things we were worried about was being able to program the mirrors to angle themselves precisely so that the laser would go back to the correct position every time," McConnell says. The solution lay in large, high-quality mirrors usually used by the defense industry to scan landscapes.

In January this year, McConnell finally took the first laser scanning confocal images of a mouse embryo using the Mesolens (see photo, above). "I was agog. It was a very elegant example that the system was actually going to work. It gave me a lot of confidence," she says. The confidence extends beyond Amos's team. "When I visited his workshop and he showed me the initial prototype and the images, I was blown away," says Rafael Yuste, a neuroscientist at Columbia University. He believes the Mesolens has potential for imaging "the activity of every neuron in the brain of an animal." Fraser adds that it will have important applications in biotech as well, such as imaging large arrays of nucleic acids or proteins.

Going it alone

The obvious next step is to develop the Mesolens into a product that can be manufactured and sold. The conventional route would be to patent the invention before taking it to an optics company for factory production. But

Not everyone thinks that's a good idea. "We need to rely on commercial enterprises to get the technology to the field," Yuste says, "by leveraging their production and distribution capabilities to lower the cost of manufacturing." Richard Ankerhold, vice president of product sectors in the BioSciences division of Carl Zeiss Microscopy, warns that small companies can face logistical problems when it comes to global support. "If there's a service call or if he has to install it outside the U.K. where he is based, it could get very difficult," Ankerhold says.

Amos argues that he can keep prices low by running his Mesolens business as close to a nonprofit as possible and relying on word of mouth for marketing. "I don't think it will cost very much more than an ordinary confocal microscope," he says, which would be about \$450,000. "My hope is that we'll be manufacturing to order next year." After all his turbulent years in the microscope industry, Amos seems to have finally found his focus.

—ANGELA SAINI

Angela Saini is a writer in London.



RADIO ASTRONOMY

Partners Prepare to Pick a Site For World's Biggest Telescope

The Square Kilometer Array will be continent-wide in scale and push technology to the limit. There are two finalists for this €1.5 billion prize

Astronomers in South Africa or Australia and New Zealand will soon be popping champagne corks in celebration of capturing one of the biggest prizes in science. It's the right to host the Square Kilometre Array (SKA), an instrument that will span a continent and comprise about 3000 radio-telescope dishes.

The winning bidder for this €1.5 billion project, which could be announced as soon as next week at a 3 April meeting in Amsterdam, will receive a huge boost to its high-tech industry and a calling card for attracting top academic talent to its shores. Not surprisingly, the two finalists in a process that began nearly 2 decades ago have been pressing the flesh and talking up their bids to representatives of the five countries that have voting authority.

Astronomers hope that the last lap in the race doesn't descend into a drawn-out political scramble that damages and delays their long-sought telescope. Indeed, those who spoke with *Science* didn't have strong views about which of the two candidate sites should be chosen. "It's very hard from a scientific perspective to choose one from the other," says Chris Carilli of the U.S. National Radio Astronomy Observatory in Charlottesville, Virginia, who co-chaired SKA's science advisory committee.

The idea for a telescope array with a total collecting area of a square kilometer originated in a 1993 workshop. The next decade

was spent building support in the community, identifying SKA's scientific goals, developing technology, and getting funding agencies on board. In 2006, four potential sites were narrowed down to two (*Science*, 18 August 2006, p. 910). Since then the candidate sites—Australia–New Zealand and South Africa teamed with eight other African countries—have been honing their bids and spending millions in the process.

Both finalists possess the two most important criteria for the site: a large, flat area that is as free as possible of any radio transmissions that might interfere with reception. "The RFI [radio frequency interference] environment is stunning at both sites," Carilli says. SKA will have a 5-km-wide core containing about half the total number of antennas—both the familiar dishes plus a new type made of arrays of static dipoles. One-quarter of the antennas will stretch out from there in five curving arms out to about 180 km. The remaining antennas will extend the arms out to more than 3000 km. Such distances are needed to get high resolution. The signals from the distant dishes are combined by computer to form images with the same resolution as a single dish the size of the whole array.

The core site of the Australia–New Zealand bid sits in the shire of Murchison in the midwest region of Western Australia. Murchison is the size of the Netherlands but with

only 110 residents. "We passed very stringent legislation to preserve radio quietness" at the Murchison site, bid leader Brian Boyle says.

The team has laid fiber-optic cable to the site, has constructed roads and buildings, and is installing renewable power supplies. The most visible demonstration of its commitment is funding a precursor telescope known as the Australian SKA Pathfinder. Its complement of 36 12-meter dishes is due to be completed this year.

The main site for the South African bid is in the Karoo region of the Northern Cape. Its farthest antennas will reside about 4500 km away in Ghana. The Karoo is, like Murchison, remote, flat, and dry. But its elevation—1000 meters above sea level compared with Murchison at less than 300—gives it a slight advantage for making observations at higher frequencies.

The bid team has also put in buildings, new roads to the site, and power and data cables. South Africa's new radio-quietness law "gives the science minister power over any development in a 500-km-diameter area around the site," team leader Bernie Fanaroff says.

The team has so far built and is commissioning an array of seven dishes: the Karoo Array Telescope (KAT-7). Its dishes are constructed from carbon-fiber composite as a test of the sort of mass-production techniques that will be needed for SKA's many dishes. A larger precursor, the 64-dish MeerKAT, is in the pipeline.

The two finalists submitted their formal bids in September, and representatives of both teams attended a question-and-answer session in December with the Site Selection Advisory Committee (SSAC), a group of 12 independent experts. The committee delivered its recommendation to the SKA organization in February.

CREDIT: SPDO/TDF/DRAQ/WINBURNE ASTRONOMY PRODUCTIONS

Downloaded from www.sciencemag.org on March 29, 2012



Antennas at the ready. SKA will deploy both the familiar dishes (opposite) and a new type made of arrays of static dipoles (left, under protective covers).

Although some 19 countries have been involved in planning SKA, only seven were ready to become members of a not-for-profit company that was set up in November in the United Kingdom to take legal responsibility for the project. The signatories have pledged €69 million of the €90 million needed for the 4-year preconstruction phase. Of these seven, the site candidates—namely, Australia, New Zealand, and South Africa—are excluded from voting on the site, leaving the final say-so to representatives from China, Italy, the Netherlands, and the United Kingdom. On 19 March, Canada became the fifth voting member.

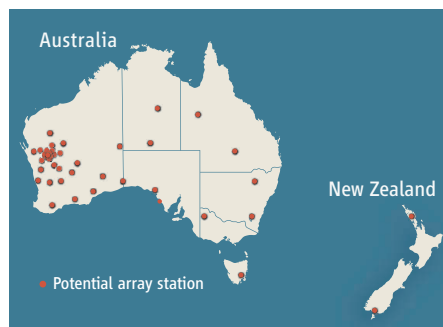
Science has not seen SSAC's recommendation, but press reports suggest that it gave South Africa a slight edge. In any case, both teams are making sure their bids are seen in the best possible light, with press releases trumpeting every milestone, however minor. South Africa announced this month that KAT-7 had succeeded in observing neutral hydrogen in a nearby galaxy (a major goal for SKA), for instance, while Australia said that signals from telescopes in Australia and Korea had been successfully combined across a distance of 8000 kilometers.

On 19 March, the SKA board discussed the site selection committee's recommendation. The voting governments are weighing the two proposals before their next meeting on 3 April, but SKA organization director Michiel van Haarlem is not expecting an immediate decision. "It's going to take as long as it's going to take," he says.

Researchers are hoping the wait won't be too long. They are eager to get started on the

next phase of the project, which is to decide the final structure of the array and produce construction-ready plans by 2016. If construction indeed begins then, observations using 10% of the total array could begin in 2020.

The SKA organization has not finalized plans for the array, in part because they depend on the site chosen but also because astronomers were waiting for technology to catch up with their ambitions. When SKA was first imagined in the 1990s, the focus was on as large a collecting area as possible. But there was no way to affordably process and store all the data. "It needed a leap in technology. The computing resources didn't exist," says Albert Zijlstra, director of the Jodrell Bank Centre for Astrophysics in the United Kingdom.



Southern exposure. Both bids rely upon access to large portions of a continent; the South African proposal involves sites in nine countries.

The initial goal of SKA was to detect light from neutral interstellar hydrogen that may allow astronomers to peer back into the "dark age" of the universe before it was populated with stars. The light is very faint, so a huge collecting area is needed. Astronomers soon realized that a telescope of that size could do other things, too. Early last decade, Carilli and Steven Rawlings, a U.K. astrophysicist who died earlier this year in unexplained circumstances, headed a panel that identified five key scientific goals: probing the dark age for the first stars and galaxies, searching for prebiotic molecules, investigating the origins of the cosmic magnetic field, testing gravity by observing pulsars around giant black holes, and studying galaxy evolution and dark energy.

These disparate targets require a multi-talented array, both sensitive (a big collecting area) and high resolution (widely spaced antennas). It will also have to span wavelengths ranging from 3 centimeters to 4.3 meters. Long wavelengths are a revived area of study for astronomers rejuvenated by recent advances in computing power. Instead of using dishes, astronomers are starting to use static arrays of simple dipole antennas on the ground.

These "aperature arrays" pick up signals coming from all directions. Using fast signal processing, computers insert slight delays between the signals from one row of dipoles compared with the next, and a further delay on the one after that, and so on. In this way the processing "steers" the sensitivity of the array in a particular direction so that it only "sees" a certain patch of sky.

The clever trick with this computer-assisted beam-forming is that an aperture array can form multiple beams and look at many parts of the sky at once. This ability makes them incredibly fast at doing surveys, something that dishes struggle with. "The survey speed will be 10,000 times faster than now," says Michael Kramer of the Max Planck Institute for Radio Astronomy in Bonn, Germany. SKA will be able to survey all the sky visible from the site once a month. That feature will allow astronomers to spot changes in the radio sky as never before.

Beyond achieving SKA's five goals, astronomers dream of discovering something completely unknown. Says Kramer: "We're pushing parameter space so much with SKA, we're bound to discover something we don't know about yet."

—DANIEL CLERY



LETTERS

edited by Jennifer Sills

Electronic Textbooks: Why the Rush?

THE RACE TO REPLACE TRADITIONAL TEXTBOOKS WITH ELECTRONIC VERSIONS IS ON. ALTHOUGH electronic textbooks have been most carefully tested in university students, the Obama Administration is advocating their use in elementary and secondary schools. In February, Secretary of Education Arne Duncan recommended that states allow school districts to spend money once reserved for textbooks on Kindles, Nooks, and iPads (1). As educational tools, electronic textbooks offer the promise of easy updates, cost savings compared with print, flexibility, and integrated features such as video, hyperlinks, and software that allows

students to collaborate. However, electronic textbook sales (2) have not followed the upward trend of e-books (3), and scientific studies of students reading and learning from e-readers suggest caution (4–11).

The difference in sales may reflect important differences in content and goals. Electronic textbooks typically present more information, much of it unfamiliar. Many e-books have a narrative structure, whereas electronic textbooks are more often structured hierarchically. Furthermore, e-books are typically read for

pleasure, whereas electronic textbooks are read for learning and retention.

Recent research shows that college students learn equally well from e-readers or printed text (4–6), but electronic textbooks carry a cost in efficiency. Reading electronic textbooks takes longer, on average, than reading print, and many students report higher levels of fatigue upon completion (7, 8). The source of this cost is unclear, but the effect is strong enough that the majority of college students prefer traditional print books when offered a choice (2, 4, 7). The preference for traditional textbooks is unrelated to previous experience with e-books, so it does not appear to be a matter of digital literacy (7).

Meanwhile, experiments with children in early grades more often show e-books equivalent to or even superior to traditional print (9). Younger children are offered simpler, narrative texts and are not asked to study and remember the content.

The features that e-book readers make possible seem like an obvious boon for e-textbooks. Surely students will learn more if they can, for example, click a hyperlink that defines an unfamiliar word, or if they can use a mouse to rotate a complex molecule in three dimensions. But years of research on computer learning shows that these opportunities can backfire. Students who click on too many hyperlinks may lose the thread of what they are reading (10). Three-dimensional figures can distract and confuse students with poor spatial abilities to such an extent that they learn better with a simple picture (11). The networking capability of e-readers is another advantage that can cut both ways. Although students can collaborate more easily, Facebook and other social media distractions are just a click away and are utilized at far higher rates while studying by students using electronic textbooks (6).

Electronic textbooks do offer substantial advantages over traditional printed text, such as the opportunity to make timely updates, adapt to learner preferences, and embed multimedia and learning activities—it's one thing to read about the fall of the Berlin Wall, but



it's quite another to see a video of it. However, research shows that students likely do not interact with electronic textbooks as they do with traditional print, and the broader research base on multimedia learning indicates that considerable care must go into the design of special features to ensure that they augment learning rather than detract from it. There is no indication that publishers are investing the time and hard work required to leverage this information into a new generation of electronic textbooks. Rather, it seems that most are taking the pedagogical devices from print books and putting them in digital format, with little evidence that they positively affect learning.

Federal Communications Commission Chairman Julius Genachowski recently said, "We absolutely want to push the process [of moving from print to electronic textbooks]" (1). If the federal government plans to push the process, it should take steps to promote the science and insist on the evidence that can ensure that electronic textbooks fulfill their potential.

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Mangrove loss. Brazil's Forest Act threatens coastal wetlands.

Protecting Brazil's Coastal Wetlands

IN DECEMBER, BRAZIL'S SENATE PASSED controversial changes to the Forest Act ("Controversial changes to forest law pass Brazilian Senate," *News of the Week*, 16 December 2011, p. 1478). Much has appropriately been said about implications of Brazil's forest code revision over Atlantic and Amazon rainforest areas (1–3), but little focus has been given to potential effects on coastal wetlands. The forest code alterations propose the conversion of up to 10 and 35% of all salt flats along Brazil's northern and southern coasts, respectively, into shrimp ponds. Salt flats are one variation of the coastal wetland ecosystem, which has different names depending on its characteristics. ("Mangroves" have woody trees, "mud flats" lack woody trees, "salt marshes" are covered by herbaceous vegetation, and "salt flats" lack herbaceous vegetation. One area may fluctuate between these states over time.) Salt flats are a vital part of threatened mangrove ecosystems, yet major mangrove mapping programs (4, 5) did not compute their total area. Without knowing the exact extension of salt flats and their effects on the mangroves, it is impossible to manage these resources responsibly. The salt flat conversions outlined in the forest code could lead to staggering mangrove losses along the northern coast, given that 57% of the country's mangroves are located in this region (6). Along the southern coast, salt flat habitat conversion could be catastrophic, considering that most of the mangrove losses to date—50,000 ha during the past 25 years—have occurred in these regions (7).

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the past 3 months or matters of general interest. Letters are not acknowledged upon receipt. Whether published in full or in part, Letters are subject to editing for clarity and space. Letters submitted, published, or posted elsewhere, in print or online, will be disqualified. To submit a Letter, go to www.submit2science.org.

Mangroves are among the most carbon-rich forests in the tropics (8), accounting for more than 50% of Earth's blue carbon sinks ("blue carbon" refers to carbon stored by coastal and marine ecosystems) and for 71% of all carbon storage in ocean sediments (9), which can remain stored for millennia. Brazil has the third-largest area of mangrove coverage in the world, including 50% of all of South America's mangroves (10, 11). Given that the destruction of blue-carbon systems results in immediate greenhouse gases emissions (12), it is clear that Brazil plays a major role in CO₂ stabilization and therefore owes an explanation not only to its own people but to the international community.

Although Brazil's forest code was approved by the Senate, we hope that President Dilma Rousseff will honor the international agreements Brazil has made by rejecting the current code's version.

ANDRE SCARLATE ROVAI,^{1*} RICARDO PALAMAR MENGHINI,² YARA SCHAEFFER-NOVELLI,³ GILBERTO CINTRÓN MOLERO,⁴ CLEMENTE COELHO JR.⁵

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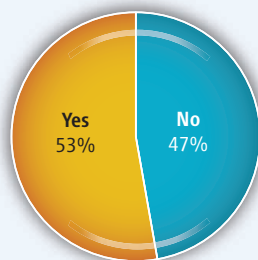
Readers' Poll Results

Bad Advice?

In the 17 February issue of *Science*, M. S. Cohen questioned the traditional advice that graduate students and postdocs should find their next position at a different institution. We asked you to weigh in by answering this question:

Does the standard practice of applying for postdoc and new faculty positions outside one's home institution make sense?*

More than 1700 of you responded, from dozens of countries all over the world. Here are your results.



Tradition's nemesis, flexibility, is what Cohen argues for, and I think he is right.

—reader Wayne Matten

You should be evaluated by your scientific expertise and not your willingness to change your home!

—reader Marten

If you stay at the same location, people begin to think alike, which stifles the advancement of research.

—reader Penny

A selection of your thoughts:

Maybe [moving locations] was the best way to network before we entered the future, but rest assured this is the future now, so I can talk to, connect with, and see pretty much anyone doing research throughout the world.

—reader Kyle McKeown

Long gone are the days where postdocs or junior faculty were well paid and spouses and children could tag along to work abroad. Dual incomes for faculty with spouses and child-raising commitments are the norm. Expecting faculty to put career above fulfilling personal life is unrealistic.

—reader Anonymous

I ... stayed in the same institution (family keeps me in the area) but switched fields completely to do my postdoc. My postdoc lab is COMPLETELY different from my Ph.D. lab. I am challenged every day and have my own expertise to offer to my colleagues.

—reader Erica

Why is a broad range of experience only required for early-career investigators? It seems to me that the folks who need the most shaking up are the tenured faculty who have been doing the same research for 20 years.

—reader Shelley

The real winner from the standard advice may be the [scientific] enterprise as a whole. Unlike private enterprise, which maximizes the profitability of individual firms, publicly funded science should maximize the performance of the entire system rather than of individual laboratories.

—reader Bruce Hamilton

What we need to avoid is the opposite scenario, where it's preferable to stay in your own institute and scientists who want to move are looked at poorly.

—reader Caroline Angelard

Positions for young scientists such as junior group leader, junior professor, and assistant/associated research professor are already available in German and Chinese top universities/institutes for those who prove themselves during their postdoc. I think this is a good idea.

—reader Nashat Abumaria

*See the poll at <http://scim.ag/GRc4gg>.

Polling results reflect those who chose to participate; they do not represent a random sample of the population.

CORRECTIONS AND CLARIFICATIONS

Reports: “An impactor origin for lunar magnetic anomalies” by M. A. Wieczorek *et al.* (9 March, p. 1212). Figure 1 did not correctly display latitude and longitude grid lines in three panels, and the ellipses outlining the South Pole–Aitken basin were missing from the left panels. The corrected figure is shown here. The figure has been corrected in the HTML and PDF versions online.

TECHNICAL COMMENT ABSTRACTS

Comment on “Disentangling the Drivers of β Diversity Along Latitudinal and Elevational Gradients”

Hong Qian, Xianli Wang, Yangjian Zhang

Kraft *et al.* (Report, 23 September 2011, p. 1755) analyzed two data sets and concluded that “there is no need to invoke differences in the mechanisms of community assembly in temperate versus tropical systems to explain these global-scale patterns of β diversity.” We show that their conclusion is based on inappropriate data and inadequate methods of analysis.

Full text at www.sciencemag.org/cgi/content/full/335/6076/1573-b

Comment on “Disentangling the Drivers of β Diversity Along Latitudinal and Elevational Gradients”

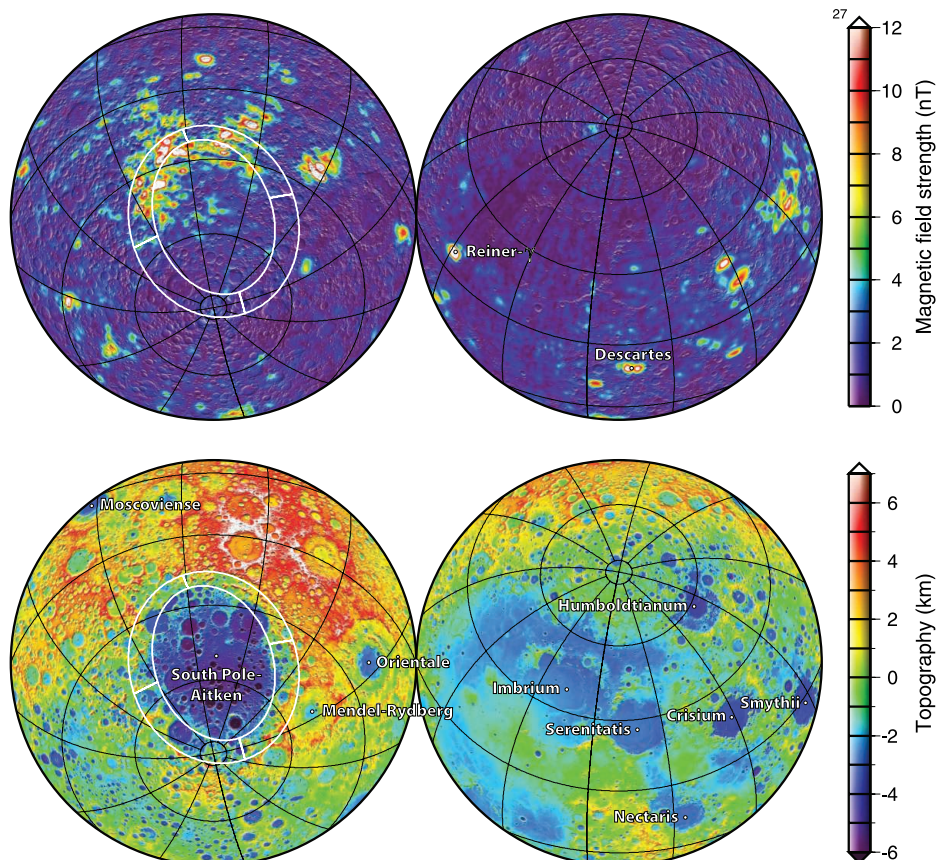
Hanna Tuomisto and Kalle Ruokolainen

Kraft *et al.* (Report, 23 September 2011, p. 1755) argued that the latitudinal trend in β diversity is spurious and just reflects a trend in β diversity. Their results depend on the idiosyncrasies of their data, especially the latitudinally varying degree of undersampling and a local sampling setup that is not suitable for analyzing drivers of β diversity.

Full text at www.sciencemag.org/cgi/content/full/335/6076/1573-c

Response to Comments on “Disentangling the Drivers of β Diversity Along Latitudinal and Elevational Gradients”

Nathan J. B. Kraft, Nathan J. Sanders, James C. Stegen, Marti



J. Anderson, Thomas O. Crist, Howard V. Cornell, Mark Vellend, Jonathan M. Chase, Liza S. Comita, Kendi F. Davies, Amy L. Freestone, Susan P. Harrison, Brian D. Inouye, Jonathan A. Myers, Nathan G. Swenson

Qian *et al.* and Tuomisto and Ruokolainen critique our analyses of elevational and latitudinal variation in tree diversity. We address their points by reanalyzing different subsets of our data and by clarifying certain misconceptions, and reiterate that gradients in β diversity can be explained in the elevational and latitudinal tree data sets by variation in the size of species pools.

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India's Path to Knowledge

DEBATE ABOUT SCIENCE AND HIGHER EDUCATION in India needs to shift away from preoccupation with bureaucratic control, the likely emergence of India as a powerhouse of science, and allocation of resources to elite universities (“India rising,” R. Stone, *News Focus*, 24 February, p. 904). Instead, the focus of the discussion should be on identifying the knowledge required to alleviate suffering from poverty, hunger, disease, injustice, and inequity; prevent environmental degradation; conserve the region's unique biological and cultural heritage; and meet developmental challenges. Such knowledge is not entirely built on the principles of natural sciences (the subject

of the 24 February *News* special section on Science in India).

To make the vision of just and sustainable societies a reality, we will need a different kind of knowledge and institutions. Minimizing red tape and increasing expenditure in science and higher education will help, but will not necessarily reduce the deficit in the type of knowledge needed.

Institutions succeed in generating relevant knowledge when they take into account the economic, cultural, and social realms of their setting. Knowledge-generating institutions in the West played an important role in developing science and technology for the benefit of their people because of the historical, political, and economic context in which they were set up, and because continent-

wide resources were available for the betterment of their people. Given that the world in the 21st century is crowded and resources in India are comparatively scarce, such institutions may not represent the best models for India to follow.

India must find its own path to knowledge generation based on its diverse beliefs, unique cultural heritage, rich and deep indigenous intellectual traditions, a vibrant civil society, and the enormous potential of its more than one billion people. In this quest for knowledge, modern science will be just one of the paths to solutions.

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Comment on “Disentangling the Drivers of β Diversity Along Latitudinal and Elevational Gradients”

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Kraft *et al.* (Report, 23 September 2011, p. 1755) analyzed two data sets and concluded that “there is no need to invoke differences in the mechanisms of community assembly in temperate versus tropical systems to explain these global-scale patterns of β diversity.” We show that their conclusion is based on inappropriate data and inadequate methods of analysis.

Understanding the mechanisms that drive β diversity—species compositional turnover among sites with respect to environmental variation and dispersal limitation—is an important step toward understanding global patterns of species diversity. Kraft *et al.* (1) related β diversity between ten 0.01-ha subunits of each of 205 transects to latitude or elevation and made a general conclusion on the mechanisms of community assembly in temperate versus tropical systems, which represent an ecological (largely temperature) gradient. Although the total area of each transect is fixed (0.1 ha) in (1), spatial extent varies among the transects because the ten 50-m-long subunits of each transect were placed in a random zig-zag pattern (2). Consequently, β diversity among transects is not comparable. In their study, each transect was sampled in such a way that a set of 10 subunits of the transect represented a relatively uniform segment of a forest community (2); environmental variation within the transect was therefore minimized. Because the largest distance separating any two subunits of a transect is usually much shorter than 500 m, β diversity between patches of a forest separated by such short distances is unlikely to be driven by environmental variation and dispersal limitation. Thus, their data cannot be used to test whether the ecological processes determining species composition differ among latitudes or altitudes.

It is widely recognized that latitude per se has little biological meaning. Statistical associations between species diversity and latitude are not directly causal but are derived from

covaried environmental variables (3), primarily temperature. Temperature, in turn, is not constant at any given latitude and can vary dramatically both among and within regions. The same temperature can be found more than 20° of latitude apart at different longitudes (4), and species distributions and vegetation zones are known to reflect this (5). Furthermore, unique historical processes can result in substantial differences in

the sizes of species pools and β diversity among regions with similar environmental conditions (6, 7). Consequently, when transects from different biogeographic regions are combined in a single analysis, as was done in (1), latitude is necessarily a poorer surrogate for the underlying environmental drivers of diversity than it would be if analyses were carried out within narrower longitudinal belts. When drawing ecological conclusions from their results, Kraft *et al.* did not take this constraint into account.

The majority of the 197 transects used in Kraft *et al.*'s (1) latitudinal analysis are located in South America. We reanalyzed Kraft *et al.*'s data points located in South America south of the equator [supporting online material (SOM) S1]. This subset data included 72 Gentry's transects and are constrained within a single biogeographic region and a unidirectional equator-pole gradient. Our results show that there is a significant latitudinal gradient of β diversity after accounting for γ diversity (Fig. 1C), contrary to Kraft *et al.*'s conclusion.

Elevations vary greatly among the 197 Gentry's transects used in (1), ranging from 20 to 2770 m (2), and the transects with high elevations are

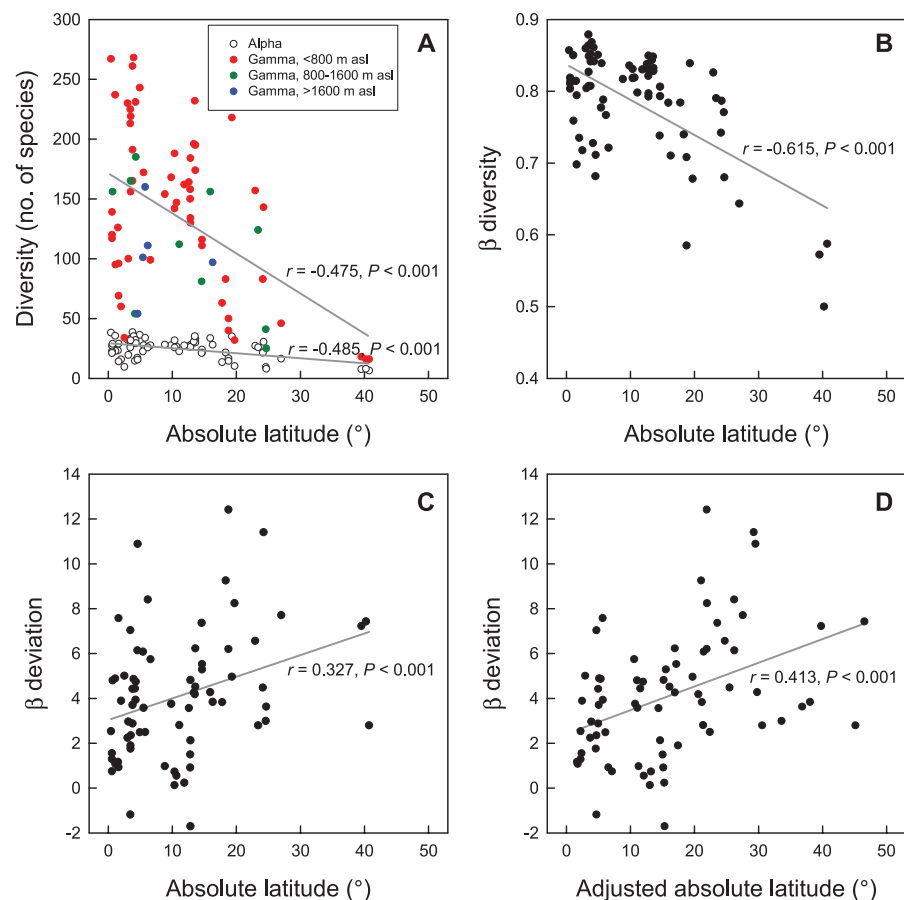


Fig. 1. Latitudinal trends in mean α and γ diversity (A), β diversity (B), β -diversity deviation without correcting for elevation difference (C), and β -diversity deviation after correcting for elevation difference (D) for woody plants in the 72 Gentry's transects in South America south of the equator (SOM S1). The data for α diversity, γ diversity, β diversity (partition), and β deviation were obtained from the corresponding author of Kraft *et al.* and were used in their figures 1 and 3.

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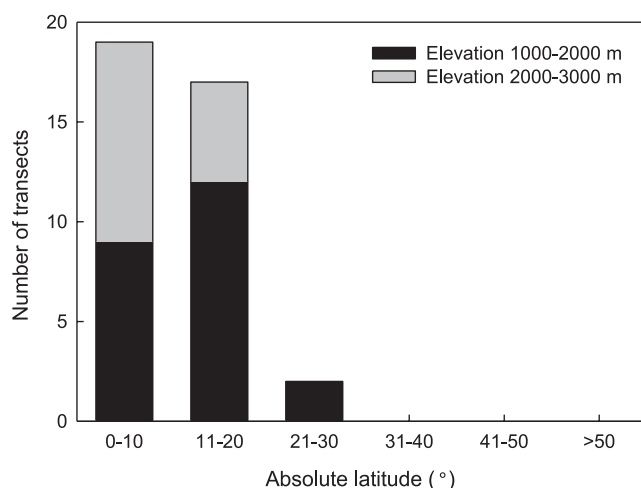


Fig. 2. Latitudinal distribution of the 38 transects at 1000 m or higher elevations in the data set of the 197 transects used by Kraft *et al.*

exclusively located at low latitudes. For example, 38 of the 197 transects are located at 1000 m or higher elevations, and nearly all of these high-elevation transects are located in tropical regions. Moreover, the majority of the transects with elevations higher than 2000 m are located near the equator (Fig. 1A and Fig. 2). The average temperature lapse rate, at which the air temperature decreases with elevation, is about 6.5°C for every 1000-m rise in elevation (4). An upward shift of 100 m is predicted to translate into a polarward shift of 100 km in the temperate zone (8, 9). This prediction is consistent with the observation that the tree line declines ~100 m for every degree of latitude (5). Thus, a transect located at 2770 m near the equator is ecologically equivalent to a transect located at ~25°S or N in latitude at a low altitude. The failure to take into consideration the altitude-latitude relationship in (1) would have disfavored species richness at low latitudes and, thus, have undoubtedly biased their results and conclusions. After adjusting the latitudes of the 72 transects of South America south of the equator by using the 100-m elevation for 100-km latitude converter in our analysis, the relationship between latitude and β diversity is reinforced, regardless of whether β partition ($r = -0.659$, $P < 0.001$) or β deviation (compare Fig. 1C with Fig. 1D) is used.

Kraft *et al.*'s main conclusion is primarily based on the lack of linear relationships in the

data presented in their figure 3C and figure 3D, where the relationships between β deviation and latitude/altitude tended to be unimodal (hump-shaped). Ecological factors that drive diversity patterns may have nonlinear relationships with latitude or altitude. The unimodal relationship shown in (1) may result from sampling and analysis biases and ecological mechanisms (SOM S2). For example, the unimodal relationship between β deviation and elevation in (1) is likely partly because their low elevation sites were located in areas with heavy human disturbances and do not represent the general conditions of forests at low elevations in the region (10) (SOM S2).

To illustrate the relationship between β diversity and latitude with γ diversity accounted for at a broad spatial scale, we analyzed a data set for woody plants (trees only) in North America (11–15). Our data set included 398 transects, each being 1100 km long and including 10 quadrats of 110 km by 110 km, which are approximately 1° by 1° latitude-longitude quadrats at the equator (SOM S3). β diversity for trees in North America is significantly and negatively correlated with latitude ($r = -0.751$, $P < 0.001$). After removing the statistical effect of γ diversity, latitude still explained a significant amount of the variation in β diversity ($R^2 = 7.3\%$, $P < 0.001$) (SOM S3).

We conclude that the failure of finding the relationship between β diversity and latitude or

elevation after accounting for the species pool in (1) resulted from the use of inappropriate data and inadequate methods. The mechanisms of community assembly that drive global patterns of β diversity remain open for investigation.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/335/6076/1573-b/DC1
SOM Text
Fig. S1
References

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Comment on “Disentangling the Drivers of β Diversity Along Latitudinal and Elevational Gradients”

Hanna Tuomisto* and Kalle Ruokolainen

Kraft *et al.* (Report, 23 September 2011, p. 1755) argued that the latitudinal trend in β diversity is spurious and just reflects a trend in γ diversity. Their results depend on the idiosyncrasies of their data, especially the latitudinally varying degree of undersampling and a local sampling setup that is not suitable for analyzing drivers of β diversity.

Kraft *et al.* (1) started with the premise that trends in β diversity are indicative of species responses to environmental gradients and claimed that the spatial scale of their data set “is appropriate to capture responses to fine-grained environmental heterogeneity.” They then cautioned that “It is widely recognized that β diversity is a simple function of α and γ diversity...and, therefore, is not independent of variation in either α or γ diversity.” To determine whether β diversity within the transects of their data set just reflects variation in γ diversity, they randomly shuffled the species identities of the tree stems in each transect and subtracted the resulting mean β diversity from that actually observed. This β -deviation value showed no latitudinal trend, so Kraft *et al.* claimed latitudinal trends in β diversity to lack ecological relevance. There are three major problems with these assertions.

First, the value of β diversity in a data set can be indicative of species responses to environmental gradients only if the sampling setup is appropriate. In particular, environmental variability within sampling units should be small relative to the environmental variability among sampling units for any species-environment relationships to be detectable in the data. If latitudinal comparisons are made, the degree of environmental variability should also be sufficiently constant among the samples representing different latitudes. Kraft *et al.* used a data set of 197 transects, each of which was established by Alwyn Gentry to quantify local species richness in a relatively uniform forest site. Gentry divided the transects into ten 50-m subunits because his delimitation tool was a 50-m tape measure, not in order to analyze within-transect heterogeneity. Indeed, there is no documentation of either the degree of environmental variability in the transects or on the spatial configuration of the subunits in relation to environmental heterogeneity or even to each other, so the data cannot be used to assess whether the ecological processes

determining species composition differ among latitudes. In effect, each subunit provides a haphazard sample of the local species pool, so it is not surprising that Kraft *et al.* found the results using Gentry's original data to be very similar to results obtained with simulated random sampling.

Second, the assertion of Kraft *et al.* that variation in β diversity is dependent on variation in γ diversity because β is a simple function of α and γ is mathematically invalid. They defined α diversity as the average number of species observed in the subunits of a transect and γ diversity as the total number of species observed in the entire transect. They used these values to calculate proportional species turnover (2) $\beta_p = 1 - \alpha/\gamma$, which they called β diversity. An increase in γ does not need to have any effect on β_p [or the other definitions of β used in the supporting online material of (1)] as long as α is free to vary: If α increases by the same factor that γ does, β remains unchanged (2–4). When all subunits are compositionally identical, β_p equals zero because then α equals γ no matter how many species are involved.

This leads to the third problem: Kraft *et al.* observed a correlation between β_p and γ and concluded that β_p is causally dependent on γ . They touched on a real and relevant issue but failed to notice that the correlation between β_p and γ is as spurious as the correlation between β_p and latitude. Both correlations are due to sampling constraints inherent in Gentry's data set, and these cause a latitudinal gradient in the degree to which α is prevented from becoming as large as γ . Both α and γ depend on the relation between the observed number of individuals and the number of species in the local species pool (Fig. 1). It has been known for a long time that compositional differences among communities are overestimated if the communities are under-sampled—that is, not all the species that are present in the community are included in the sample

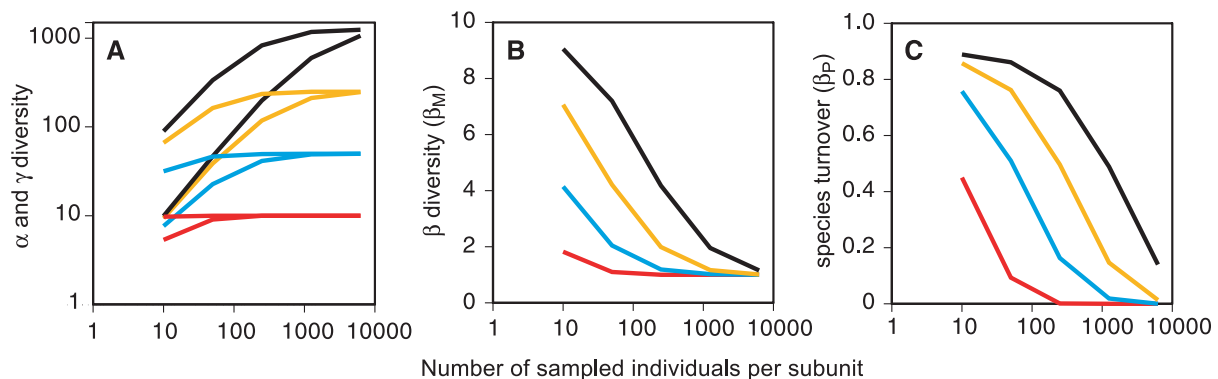


Fig. 1. The dependency of α and γ diversity (A), β diversity (B) and proportional species turnover (C) on species pool size and within-subunit number of individuals in 10-subunit data sets. Each color corresponds to a different species pool size: red, 10 species; blue, 50 species; orange, 250 species; black, 1250 species. Each line shows the mean of 1000 replicates where 10 subunits of either 10, 50, 250, 1250, or 6250 individuals were randomly drawn from a log-normal species-abundance distribution. In (A), the lower line for a given species pool size

shows α diversity and the upper line, γ diversity. With a sufficiently large number of individuals, α and γ converge; all subunits were drawn from the same species pool, so any compositional differences among them are caused by under-sampling. In (B), the factor by which γ exceeds α ($\beta_M = \gamma/\alpha$) decreases with the increasing number of individuals sampled. In (C), the proportion of species in the entire set of 10 subunits that does not fit within a single subunit ($\beta_p = 1 - \alpha/\gamma$) decreases with increasing number of individuals sampled.

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representing the community (5–7). If the local tree species pool is small (a handful of species, as in high-latitude areas), a few dozen tree stems are sufficient for all of the relevant species to be present in a subunit. However, in tropical forests, a single site can contain more than 1000 tree species (8), so each subunit has to contain thousands of stems to be representative of the local community (Fig. 1) (9). Gentry's subunits had an average of just 34 stems, so the degree of undersampling in the data is strongly correlated with latitude, which forces observed β diversity to correlate with latitude as well.

Such results on latitudinal trends are dependent on the properties of the data set used and should not be interpreted as if they were valid for β diversity in general (10). Of course, latitudinal trends in undersampling are not only a problem of local-scale studies. Macroecological studies are also affected, because the availability of field observations on species occurrences is in general

poorer in tropical areas than at higher latitudes, especially if related to the size of the regional species pool (10–13).

Although Kraft *et al.* titled their Report “Disentangling the drivers of β diversity along latitudinal and elevational gradients,” they used an example data set where the only important driver of β diversity is the degree to which sampling units fail to provide adequate representation of the local species pool. As a consequence, their results shed no light on whether there are latitudinal gradients in community assembly processes or ecological mechanisms driving β diversity. These questions remain unanswered until they are addressed with data sets that provide an adequate, documented sampling of within-site environmental variability in addition to solving the under-sampling problem.

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Response to Comments on “Disentangling the Drivers of β Diversity Along Latitudinal and Elevational Gradients”

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Qian *et al.* and Tuomisto and Ruokolainen critique our analyses of elevational and latitudinal variation in tree diversity. We address their points by reanalyzing different subsets of our data and by clarifying certain misconceptions, and reiterate that gradients in β diversity can be explained in the elevational and latitudinal tree data sets by variation in the size of species pools.

Both Qian *et al.* (1) and Tuomisto and Ruokolainen (2) critique the Gentry data sets. Qian *et al.* state that β diversity among Gentry subplots is not comparable across locations because the spatial orientation of the subplots varies among locations. Subplot spatial orientation varies haphazardly across sites, and no systematic trends in spatial orientation exist. Therefore, gradients in Gentry plot β diversity are neither biased nor invalid. Qian *et al.* also assert that because the Gentry subplots tend to be located to minimize coarse environmental variability among subplots at a location, they cannot be used to study the ecological processes determin-

ing species composition. We originally stressed that the Gentry data are not appropriate for testing how coarse-grained environmental heterogeneity structures communities among subplots within a location (3). We disagree, however, that the scale of the Gentry plots makes them inappropriate for studying the myriad processes that structure community composition. Indeed, considerable β diversity exists within each location in the Gentry data estimated by the β partition either before [figure 1C in (3)] or after [figure 3C in (3)] implementing our sampling-based null model. This second point is a misunderstanding shared by both Qian *et al.* and Tuomisto and Ruokolainen. Specifically, after conditioning the observed β diversity within each location on location-level γ diversity, we still find extensive and substantial nonrandom patterns of species turnover at all points along both latitudinal and elevational gradients [i.e., the β deviation was >0 for almost all points in figure 3C and for all points in figure 3D in (1)]. Our key result, therefore, is not that species co-occurrence patterns can be explained by a null model, as Tuomisto and Ruokolainen state. Instead, we find that the β partition shows no trend with latitude or elevation after accounting for γ diversity with an appropriate null model. Although broader-scale sampling at each location might capture β diversity driven by coarser-grained environmental factors, there is pervasive, nonrandom β diversity at the spatial scale measured by the Gentry data. Our paper does not state that “latitudinal trends in β diversity...lack ecological relevance,” as Tuomisto and Ruokolainen suggest. Nonrandom patterns in the smaller-scale turnover that we documented are both real and ecologically relevant.

Qian *et al.* critique our use of latitude instead of temperature and suggest a correction to latitude that accounts for plot elevation as a proxy for mean temperature (1). We question the va-

lidity of this simple correction, given that many factors besides mean temperature differ among locations. Nevertheless, when we apply this adjustment to the full 197-location data set, the results agree with those of our previous analysis (3). Specifically, we find that the negative correlation between latitude and β diversity can be explained by our null model in the original data [Fig. 1, A and B, after (3)] and after adjusting latitude for elevation in the manner proposed (Fig. 1, C and D). We reach the same conclusion when we remove high-elevation sites from the analysis [Fig. 1, E and F; defining high elevation as >1000 m after figure 2 in (1)].

Qian *et al.* examined whether β -diversity patterns in one particular subset of the Gentry data differ from patterns seen in the entire data set. We disagree that any nonrandom subset of a larger data set would be expected to show the same pattern as the entire data set. Nevertheless, if we restrict our analysis to include only the Gentry plots from the New World (158 out of 197 locations), our original conclusions are upheld, regardless of whether we adjust latitude for elevation following (1) or remove high-elevation sites (Fig. 2). However, if we reduce the data set further, as Qian *et al.* have done, to include only the 72 South American locations south of the equator (37% of the full data set, thereby excluding $>50\%$ of the New World data and 33% of the South American data), a statistically significant relationship between the β deviation and latitude emerges. What is surprising is that the pattern is the opposite of what is expected. Specifically, although β diversity for South America south of the equator is highest at the equator ($R^2 = 0.38$) [figure 1B in (1)], after correcting for variation in γ diversity, the β deviation is highest in southern South America ($R^2 = 0.11$) [figure 1C in (1)], effectively reversing the gradient. Our global analysis demonstrated how differences in β diversity across a broad gradient can be explained by differences in γ diversity, resulting in no gradient in β diversity across latitude. By focusing on a nonrandom subset of the data, Qian *et al.* show that the effect of γ diversity can be so strong that, once it is accounted for, the pattern along the latitudinal gradient is actually reversed. This gives further strong evidence for the importance of the null-model-based approach that we have developed for analyzing β -diversity trends (3).

We fully support attempts to apply our null-model approach to other data sets, as Qian *et al.* attempt to do with a new North American tree data set. Importantly, however, our null-model approach requires data on individual abundances within subplots, because it operates by randomizing individuals among samples. The additional data set does not include abundance information, so unfortunately cannot be analyzed using our null-model approach. Qian *et al.* propose to instead account for γ diversity using least-squares multiple regression. This approach fails to properly

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account for the differences in γ diversity among transects [supporting online material (SOM) S2], and their implementation suffers from important

statistical errors (SOM S3), making their approach unsuitable for analyzing the effect of γ diversity on β diversity.

Furthermore, we question the interpretation of results offered by Qian *et al.* from the North American tree analysis. Specifically, they estimated

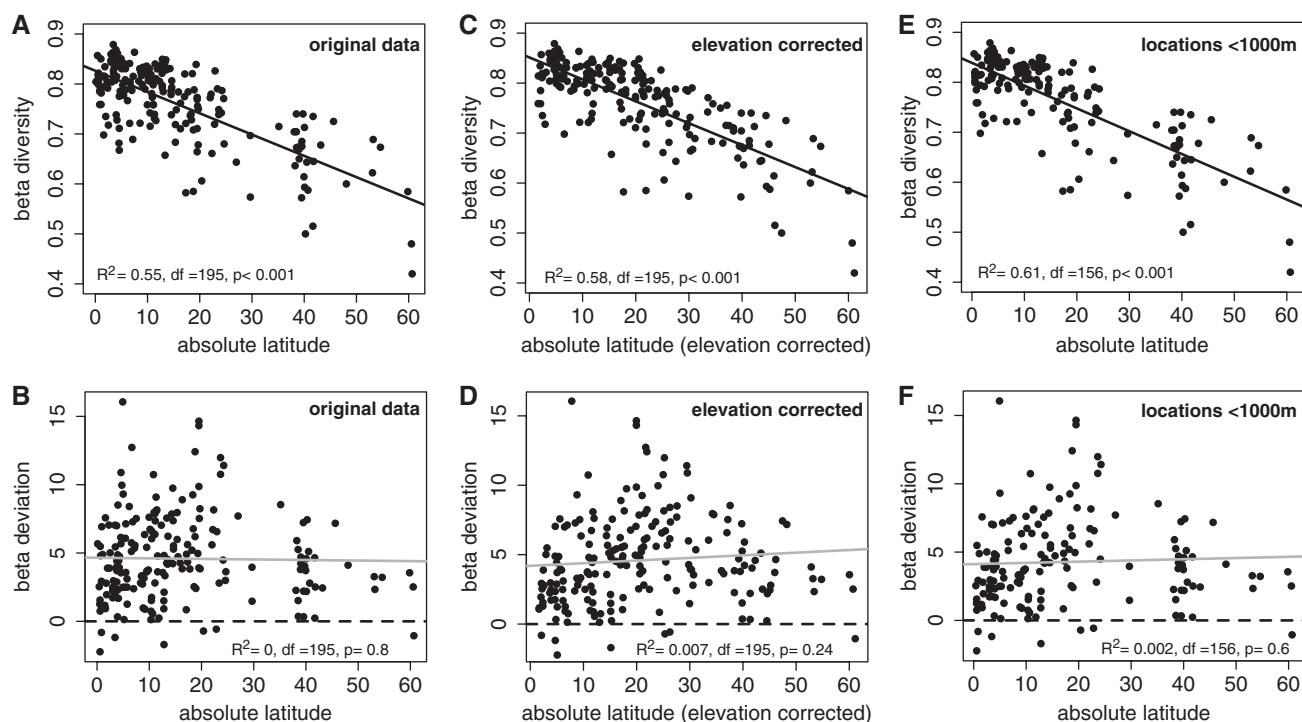


Fig. 1. Latitudinal comparison of β diversity (measured as the β partition) with the β deviation from our null model. (A) and (B) show the original results from (1), including all 197 locations across the globe. (C) and (D) adjust latitude for

elevation following the correction Qian *et al.* propose. (E) and (F) remove locations above 1000 m of elevation. Dashed line indicates a β deviation of 0. Solid lines show linear relationships; black lines are significant, gray lines are nonsignificant.

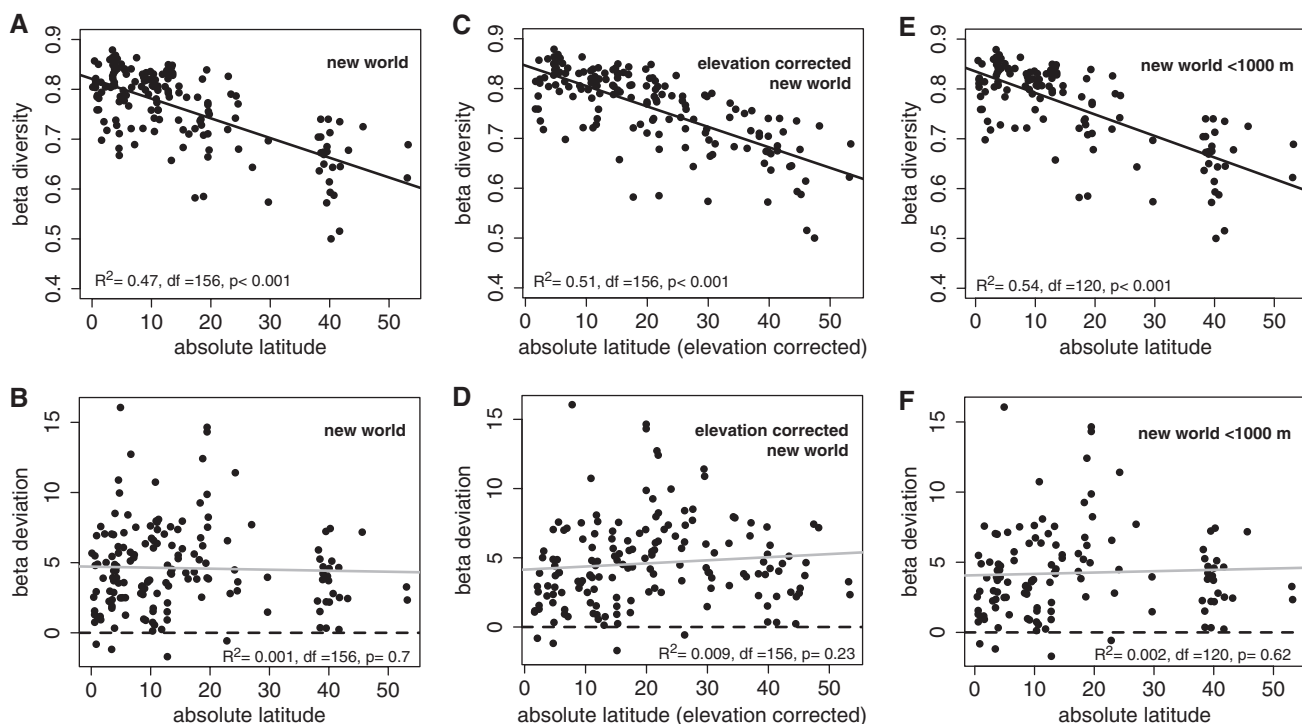


Fig. 2. Same analyses as Fig. 1, restricting data to just the New World. (A and B) β diversity and β partition versus latitude for all New World sites. (C and D) β diversity and β partition after correcting latitude for elevation using the correction Qian *et al.* propose. (E and F) β diversity and β partition after removing sites above 1000 m.

that the percentage of variance explained by latitude drops from 56.4% with β diversity measured as the β partition to just 7.3% after taking the residuals of the β - γ relationship. This is a large decrease, and although we emphasize that their method is not appropriate for their aims of accounting for the influence of γ on β , their results are nevertheless largely consistent with our main conclusion: namely, that significant variation in β diversity across large biogeographic gradients is likely to be driven by sampling effects (3).

Tuomisto and Ruokolainen question our conclusion that variation in β diversity can be driven by variation in γ diversity, claiming that our conclusion is a by-product of the fact that α diversity is not free to vary in our analyses. However, this assertion is not correct. To clarify, in our simulations, we constrained γ diversity, the number of individuals per sample, and the existing abundance distribution of the species within each location [see R code in the SOM of (3)]. Therefore, in both the simulations and the null model used to calculate the β deviation, α diversity is free to vary. Our results [e.g., figure 2A in (3)] show that increasing γ diversity does indeed drive an increase in β diversity under our random sampling null model.

Next, they propose that should the special case arise where α and γ diversity increase by exactly the same factor, β diversity would be constant with increasing γ diversity. Although this is true in their hypothetical scenario, it is clear that in both the real data sets [compare slopes in figure 1, A and B, in (3)] and in our simulations of randomly assembled communities, α and γ do not increase by the same factor. Specifically, as γ diversity increases, α diversity increases too, but by a smaller factor, yielding the increase in β diversity. Therefore, although it is theoretically possible for α and γ to increase by precisely the same factor, and it may be possible to conceive of a hypothetical scenario in which an increase in γ diversity does not increase β diversity, neither the original data sets in our analysis nor the null-model randomizations exhibited this proposed behavior of perfect scaling between α and γ , nor would we expect this to occur generally for empirical ecological systems.

Tuomisto and Ruokolainen suggest that the correlation between β and γ diversity in our data set is spurious. They show simulations indicating that α and γ depend on the number of species in the local species pool (which we define as γ diversity in our analyses) as well as the number of stems. This is exactly the point that we make in our manuscript: for example, figure 2A in (3) documents the expected relationship between α , γ , and the number of individuals in the local community under random sampling. This sampling relationship is at the core of our analyses and results (3).

Tuomisto and Ruokolainen also state that we are “undersampling” the local community and that this undersampling bias covaries with latitude in a way that drives our results. This claim seems to hinge on the idea that there is a single “best” scale at which to study ecological phenomena. We strongly disagree, as there is a long history in ecology of noting the scale-dependence of various processes (4–8). We emphasized in our paper (3) that any inferences drawn from an analysis are conditioned on the scales used in the study. As Gentry and colleagues cataloged every woody stem in each subplot, we fail to see how the data have undersampled the communities about which we draw our inferences. The solution suggested by Tuomisto and Ruokolainen to avoid this undersampling is to increase the size of local subplots until they can contain enough individuals to represent all species present in the region. This effectively requires that α and γ be measured at the same scale, but in all studies of β diversity, the scale at which α is measured is necessarily smaller than the scale at which γ is measured. Furthermore, their proposed solution would require different-sized sampling units in regions having different values for γ . This would confound sampling-scale differences with regional differences, thus making robust comparisons impossible. This also places unrealistic constraints on the spatial scale that can be considered to be a community, which in turn severely constrains the spatial scale at which one can make ecological inferences. In contrast, we have directly incorporated the sampling relationship between α and γ (in terms of the number of stems) into the null model in order to calculate the β deviation, and

this approach can be applied at any spatial scale that is of interest.

Qian *et al.* and Tuomisto and Ruokolainen suggest that the aim of our paper was to explain the effect of environmental variation on species composition. In fact, our only goal was to test the idea that the observed trends in β diversity (among subplots) with respect to either latitude or elevation could arise by random sampling from the pool of individuals at the location level. Here, we have shown that our results are robust. We welcome the application of our null-model approach to other suitable data sets, for other taxonomic groups of organisms and at other scales. As one recent example, a new global analysis of independent large-scale forest data sets implementing a similar null model to the one we developed concluded that global patterns of β diversity are largely driven by variation in γ diversity (9), much as we report (3). Proposals regarding new tests of specific mechanisms that might drive α , β , and γ diversity at a variety of scales are due to unfold but will be challenging to assess from observational data alone. A more complete understanding of what drives spatial variation in biodiversity will require, in the first instance, demonstration of the inadequacy of null models of community assembly alone to account for empirical patterns.

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NATURAL HISTORY

Fungal Fruiting Bodies and Fanatics

Linnaea Ostroff

There was never a March Hare, a Cheshire Cat, or a hookah-smoking caterpillar. The mushroom upon which the caterpillar sat, however, is real. The iconic *Amanita muscaria*, with its red cap and festive white polka dots, contains hallucinogens that cause the size of objects to appear distorted. Lewis Carroll was not unfamiliar with the effects of *A. muscaria* at the time he wrote *Alice's Adventures in Wonderland*, and mushroom intoxication is one of the few of Alice's experiences that an intrepid reader may replicate.

Mushrooms often travel from reality to mythology unchanged. Their more theatrical activities—such as appearing and disappearing instantaneously, glowing in the dark, and causing spectacular bodily harm, rapid death, and profound psychosis—fall within our definition of magic. Not classified as animals, vegetables, or minerals, mushrooms are often relegated to the world of fairies and elves. Nicholas Money and Eugenia Bone devote their recent books, *Mushroom* and *Mycophilia*, to the standing of mushrooms in nature and in human culture.

For Money, a mycologist at Miami University, the discoveries of his field are a point of pride. He offers preemptive reassurance that the magic of mushrooms will not be spoiled by revelation of their tricks, which he explains in some detail. Bone, a New York–based food writer, supplies a memoir of her passage into the insular community of the professionally and recreationally mushroom-obsessed. Neither author expects the topic to be taken with an entirely straight face. As Money puts it, “Mushrooms are never going to attract the same kind of reverence as polar bears and other charismatic megafauna.” Most of the facts are delivered with a wink, and both authors reveal their passion for mushrooms with a sheepishness that is unusual in nature writing. But mushrooms are unusual.

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Mushrooms are the sex organs of fungi. They are ballistics experts that emerge when the fungus is ready to reproduce, launch spores by the billion, and vanish. Or rather, they puff

up and deflate. The sudden appearance of mushrooms on a lawn or under a log, like many illusions, is achieved with extensive advance setup and hydraulics. After a spore germinates, it sends filaments out underground in all directions in search of food and other fungi. When two fungal colonies—or three or more, as fungi are substantially less constrained than animals—of the same species meet, their cells merge and their DNA combines in the mushroom version of mating. New spores are produced, and the cells of

the future mushroom are organized around them. This process occurs at the tips of the filaments, accounting for mushrooms' quirk of appearing in rings. When the conditions are right, water rushes in and pressurizes the assembly, swelling the cells and inflating the mushroom. In many species, that takes only a few hours, the spores are soon released, and the mushroom shrivels by sundown. Others survive a week or more, and some tougher forms may last for months.

The environmental conditions that trigger mushroom formation are highly variable and

not well understood, and a mushroom colony may spread stealthily underground for acres before fruiting. For those inclined to hunt mushrooms, their unpredictable appearance and short half-life only amplify the thrill of discovery. Bone's account includes substantial anthropological descriptions of mushroomers, who she categorizes as masters of the forest, world's leading experts, off the gridders, and belly feeders (the group in which she places herself). Habits in this community include chewing toxic mushrooms for bragging rights and hiding baskets while foraging so as not to alert the mushrooms.

Eccentric as this sounds, it can be hard to resist ascribing a degree of agency to mushrooms, particularly when their ability to sicken and kill is considered. Perhaps only the pufferfish rivals the mushroom in packing such a death risk into a small delicacy. Some poison mushrooms, such as death caps (*Amanita phalloides*), destroying angels (*A. bisporigera*), and webcaps (*Cortinarius speciosissimus*), may not cause symptoms for up to two days, at which point the liver, kidneys, and often the victim die. In contrast, convulsions can occur within minutes of eating a *Russula subnigricans*; muscle breakdown and, ultimately, heart failure follow. Many other mushrooms cause their victims to be wretchedly, albeit temporarily, ill, and all of these can be confused with edible species. With the art of identification rare and vanishing, it is a testament to the allure of mushrooms that so many people continue to play Russian roulette in the forest.

Risking death for a delectable meal may have its charms, but it doesn't entirely explain the attraction. Mushrooms can also bring you closer to god. Psilocybin, the hallucinogen in mushrooms of the genus *Psilocybe*, can produce euphoric spiritual experiences in which users report feeling at one with the universe. Although magic mushrooms have been marginalized (and illegal) since the 1960s due to their recreational use by Timothy Leary and his followers, there is now interest in using psilocybin to treat depression and various other psychiatric and neurological disorders.

Unlike psilocybin, which interacts with serotonin receptors, the active substances in *A. muscaria* act on the more fundamental *N*-methyl-D-aspartate and γ -aminobutyric acid receptors. Intoxication with ibotenic acid and muscimol is reportedly not as pleasant but apparently equally if not more enthralling. *A. muscaria* has been used for shaman-

Mushroom

by Nicholas P. Money

Oxford University Press, New York, 2011. 221 pp. \$24.95. ISBN 9780199732562.

Mycophilia

Revelations from the Weird World of Mushrooms

by Eugenia Bone

Rodale, New York, 2011. 368 pp. \$25.99, C\$29.99, £16.60. ISBN 9781605294070.



Makes you smaller. Intoxication from eating the fly agaric mushroom (*Amanita muscaria*) makes objects seem much larger than they actually are.

istic purposes for millennia, a practice that continues in Siberia.

Of course, evolution did not craft the various flavors, poisons, and psychedelics of mushrooms in accordance to human responses to them. Rather, this pharmacopoeia most likely developed to influence predatory and

sympiotic insects and microorganisms, not large predators with foraging baskets.

For being nothing more than fruiting bodies of certain fungi, mushrooms possess considerable mystique. They would be a dry subject if we used them only as food or to get high or if they were only toxic fruits.

Instead, as Bone and Money demonstrate, they can affect people in many ways—we can be drawn to mushrooms through interests in science, cooking, religion, or the aesthetics of nature. It's the vastness of their repertoire that's unnerving.

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BROWSINGS

Sustainable Materials: With Both Eyes Open. Julian M. Allwood and Jonathan M. Cullen. UIT Cambridge, Cambridge, 2012. 284 pp. £45, \$89.95. ISBN 9781906860073. Paper, £24.95, \$49.95. ISBN 9781906860059. PDF file available at <http://withbotheyesopen.com/>.

With all the attention given to nanomaterials, it is easy to forget that far more is built with steel and aluminum. These materials have large raw-material and energy costs associated with their extraction and processing, and those issues are likely to become more prominent as nations further their development. To create a sustainable future requires more than just an expectation that manufacturers and end users will independently become more efficient. Rather, further growth in the widespread use of resource-limited materials can only be developed by considering their entire life cycles, from the obtaining of raw materials through the recycling of no-longer-needed products, and with the involvement of all interested parties. Julian M. Allwood and Jonathan M. Cullen take this approach in their evaluations of specific materials, with a particular focus on steel and aluminum alloys. Mindful of both basic research and engineering, they discuss how these materials were first developed, subsequent innovations, and the processing steps and energy required to make them. They also consider ways to reduce their use, better recycle them, or replace them entirely—and whether such changes might become widely implemented. The authors focus on metallurgy. Because of the limited opportunities or immense complexities associated with concrete, plastics, and paper, they examine these classes of materials only in a single, relatively short chapter. The book offers perspectives from fundamental materials science, process engineering, product design and architecture, and economics not seen in most materials texts.

—Marc Lavine

Fundamentals of Materials for Energy and Environmental Sustainability. David S. Ginley and David Cahen, Eds. Cambridge University Press, Cambridge, 2011. 771 pp. £65, \$99. ISBN 9781107000230.

"Oh, you can't pass heat from a cooler to a hotter" state, according to the British comedy duo Flanders and Swann in their discussion of the first and second laws of thermodynamics. But we do this all the time, when we put an object in a refrigerator and the heat from that object is transferred to its surroundings. At the heart of this trick—as well as the cooking of our food, the heating of our homes, our ability to travel long distances, and the powering of all our electronic devices—lies the availability of energy in easy-to-use and (fairly) affordable forms. Increasingly, though, our supplies of energy are becoming more costly, raw material limitations are a greater concern, and the focus on dealing with pollution has intensified. We know that these issues will only worsen as the world population grows and raises its overall standard of living. The comprehensive textbook edited by David S. Ginley and David Cahen provides a tour of all aspects related to energy production and usage along with the development of new materials for generating, storing, and



Creative reuse. However, western Nebraska's Carhenge does not provide "a future business model" for recycling automobiles.

transporting energy. The authors' discussions span the range of existing technologies, from the use of fossil and nuclear fuels, through solar and wind power, to the more efficient recovery of waste heat and energy. Although the book notes the challenges that come from political and economic considerations, it devotes more attention to the assessment of the many fundamental basic materials challenges that still exist. While it is clear that we can't just burn our way to a sustainable energy future, it remains uncertain which alternatives will gain wide-scale adoption. This text does an excellent job mapping out the many pathways that are currently under exploration.

—Marc Lavine

Stem Cells: A Very Short Introduction. Jonathan Slack. Oxford University Press, Oxford, 2012. 144 pp. Paper, £7.99, \$11.95. ISBN 9780199603381. Very Short Introductions.

For all the public interest and press coverage stem cells and their "promise" have received, there should be widespread understanding of the topic. However, one finds that considerable confusion still surrounds definitions, current uses, and therapeutic potential. In this Very Short Introduction, developmental biologist Jonathan Slack provides much more than a primer. He offers clear definitions and carefully distinguishes among the various types of stem cells (embryonic, adult, or personalized; totipotent, pluripotent, or multipotent). But beyond that, he places scientific advances in historical perspective and takes pains to explain the basic biological concepts and model systems, associated technical advances, and current clinical practices (whether rational and regulated or "aspirational"), as well as areas of research controversy. Presenting accurate descriptions and appropriate technical terminology in an accessible style, his account will inform nonscientists, while researchers already familiar with the topics will find themselves largely satisfied. The book should reach the author's stated goals: Its contents will assist readers in judging media reports, increase their skepticism of claims of miracle cures, and help those in business or politics make "sensible decisions about the funding and regulation of stem cell research in the next few decades."

—Beverly Purnell

To Protect Human Subjects, Review What Was Done, Not Proposed

Robert Klitzman^{1*} and Paul S. Appelbaum²

The Advance Notice of Proposed Rulemaking (ANPRM) released in 2011 by the U.S. Department of Health and Human Services (HHS) (1) recommends many important changes to federal regulations on protection of human research subjects. Perhaps most important, through the 74 questions it poses, it offers the opportunity to rethink approaches to research oversight. The current regulatory model of prospective review, based on what researchers say they plan to do, focuses the attention of Institutional Review Boards (IRBs, which must approve proposed research) and researchers on perfecting protocols and consent forms rather than interacting with subjects. Such a regulatory model may discourage innovation in human subjects protection. In contrast, we describe how a system based on retrospective, auditlike review of a subset of projects could stimulate assessment of the effectiveness of current approaches and the development of creative alternatives, with efficiencies for all concerned.

Prospective versus Retrospective Review

Oversight of human subjects research in the United States grew out of scandals of the 1960s and early 1970s, culminating in the current U.S. regulations in 1991 (“the Common Rule”). Other countries have developed similar rules (2). Characteristics typical of a prospective approach, however, have contributed to widespread dissatisfaction with human subjects protection (3, 4). Because prospective review can only focus on what researchers say they will do, IRBs inevitably concentrate most of their attention on the minutiae of protocols and consent forms rather than on monitoring actual performance (5). Counterproductively, researchers often fine-tune their IRB submissions rather than improve their interactions with research subjects. Paperwork burdens for researchers have grown, even as studies reveal deficits in

subjects’ grasp of the projects in which they have enrolled (6, 7).

Other systems of oversight, however, have evolved quite differently and rely on retrospective review. Examples in the United States include audits of government-funded health-care programs (Medicare and Medicaid), accreditation of hospitals, Data Safety Monitoring Boards’ assessment of patient safety and treatment efficacy data during clinical trials, federal and state tax collection audits, and the tort system. None of these is exactly comparable to the task of human subjects protection. But all focus reviews on a small proportion of cases flagged as potentially problematic or selected at random, which reduces burdens for the system and those subject to its oversight (8–11).

It is understandable why, in the 1970s, the U.S. Department of Health, Education, and Welfare (parts of which were later reorganized as HHS) embraced a prospective regulatory approach. Public reaction to the revelations of abuses by researchers at Tuskegee and elsewhere called for immediate action. The possibility that additional harms might accrue to human subjects threatened the viability of medical research as a whole. Prospective review—essentially requiring investigators to obtain a permit to perform a study—appeared to be the best means of bringing the system rapidly under control. But over the long run, prospective review may be a suboptimal strategy for the oversight of much human subjects research.

Ill-Suited for Research

Prospective review is most easily applied to relatively clear-cut determinations with predictable outcomes. However, little about human research oversight is particularly straightforward. IRBs often face difficulties defining, interpreting, and applying critical concepts embodied in the regulations and central to human subjects protection (e.g., “justice” and “autonomy”). Regulations prohibit “undue inducement” (i.e., disproportionate payments to encourage participa-

tion), but IRBs and ethicists range widely in their views of how much money is “too much” (12, 13). Perceptions of “risk” are highly variable, even for common procedures (14).

Moreover, IRBs have different thresholds for approving studies and struggle with whether protections for subjects are “good enough” (14). IRBs often disagree in reviews of the same study at multiple sites (15–18). The ANPRM asks whether new regulations should standardize the topics to be included in consent forms. But consent forms involve



subjective judgments, concerning not just what information to include but how to describe a study. Many IRBs feel they play a vital role in rewording consent forms, because researchers may downplay risks and overemphasize potential benefits. But IRBs “wordsmithing” the contents differently produces inconsistent results (19).

IRBs generally argue that differences among them reflect local community values and thus are justified, but these variations often appear instead to reflect personality or institutional factors (20). Variations occur even within single institutions and IRBs over time (20). These complex, dynamic factors, not only formal regulations, affect how IRBs interpret and apply guidelines.

Although many of these issues might arise in any system of oversight, whether prospective or retrospective, the inherently speculative nature of prospective review exacerbates variability and subjectivity across IRBs. When no one can know whether subjects

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will understand phrases in a consent form, IRB members lack firm grounding for many judgments. In contrast, retrospective review encourages greater focus on events that have transpired, rather than those merely imagined and feared. At present, once a study is approved by an IRB, an investigator is generally not required to monitor or improve the effectiveness of the consent process or subjects' reactions to participation. But the possibility of being audited on the basis of how well subjects understood the study or whether they were distressed by the research procedures—based on objective, validated questionnaires—would provide different incentives. Investigators would be encouraged to try new approaches to improve the quality of interactions with subjects. Social and behavioral research, now often hostage to IRB debate over whether participants will be upset by a particular item on a questionnaire (21), should find retrospective review particularly facilitative. As data accumulate on more successful strategies for obtaining consent and avoiding risk, they can be shared with the research community.

A Path Forward

As the U.S. government reconsiders human subjects regulation for the first time in more than 20 years, thoughtful consideration of possible approaches is critical. We cannot simply turn the clock back to 1966, when prospective review was first introduced, and cast it aside. Indeed, for studies with substantial risks to subjects and uncertain likelihood of benefit, prospective review may be desirable (22, 23). But there are clearly ways to begin to integrate retrospective review into the current oversight process.

The ANPRM proposes an important step in this direction, involving a shift to retrospective assessment for certain minimal risk research, which would be excused from prospective IRB review. Investigators would decide for themselves whether their research meets the appropriate criteria, in which case they would merely register their studies with IRBs, complete a simple (approximately one-page) form, and then conduct the research. IRBs could retrospectively audit some protocols to ensure that the investigators' discretion to define their projects as minimal risk is not abused. Given that even IRBs disagree about definitions of minimal risk (16), some divergence of opinion between IRBs and researchers is to be expected, without necessarily indicating errors on either side. Although the ANPRM does not specify the consequences of negative results of an audit, we envision imposition of closer monitor-

ing and perhaps prospective review of that researcher's future studies. Careful assessment of the consequences of the changes we describe here is essential.

Of note, an increasing number of institutions have already created mechanisms for selectively auditing studies and thus have some experience with the model. If audits are already being introduced under the current retrospective review framework, why is still more fundamental reform needed? Grafting some degree of retrospective review onto the current process would not address the system's inefficiencies, including the work and delay inherent in universal prospective review, the undue weight given to written descriptions of procedures rather than actual researcher behavior, and the emphasis on speculative outcomes. Further levels of review can always be added, but each one carries costs in terms of funding, effort, and delay. Shifting toward a retrospective review model will allow finite resources to be used in a more efficient, and perhaps more effective, oversight process.

Retrospective review of minimal risk research also offers an opportunity to create an appellate IRB process, a possibility raised by the ANPRM. Currently, IRBs bear no costs if they unnecessarily nitpick a protocol or interpret regulations idiosyncratically. Each IRB acts as its own appellate court; researchers' only recourse is usually to the very body that challenged their approach. Although an appeals process could be constructed in a prospective review system, a retrospective system would allow determinations based on evidence of what actually occurred, rather than fears of what might happen. That difference may increase researcher willingness to pursue an appeals process. Regional appellate IRBs could be established by the Office of Human Research Protections, with their number depending on the volume of appeals. Due process is so fundamental a right in modern democracies that there seems little reason to deny it to researchers.

If initial moves away from a strictly prospective model are successful, it may be possible to expand them progressively to studies that pose higher levels of risk. To be sure, as researchers assume these responsibilities, they may require additional education in subject protection and research ethics. We could imagine further exemptions from prospective review for studies that certify compliance with standards, such as currently proposed for federal regulations on privacy of health information. In this way, research oversight would increasingly become a retrospective review process, sparing investiga-

tors and IRBs alike the burden of reviewing all aspects of every study in advance.

A Willingness to Rethink

The ANPRM proposes many potentially valuable alterations to the current structure of human subjects oversight, indicating a willingness by HHS to rethink what has been accepted for decades. But the most innovative aspect of the proposed changes is the signal that HHS may not be firmly wed to prospective review. We have raised here more questions than we can answer in this relatively short space, but hope that this discussion can spur analysis and debate. In these possibilities lie important hope for substantial improvement in protecting human subjects and facilitating research.

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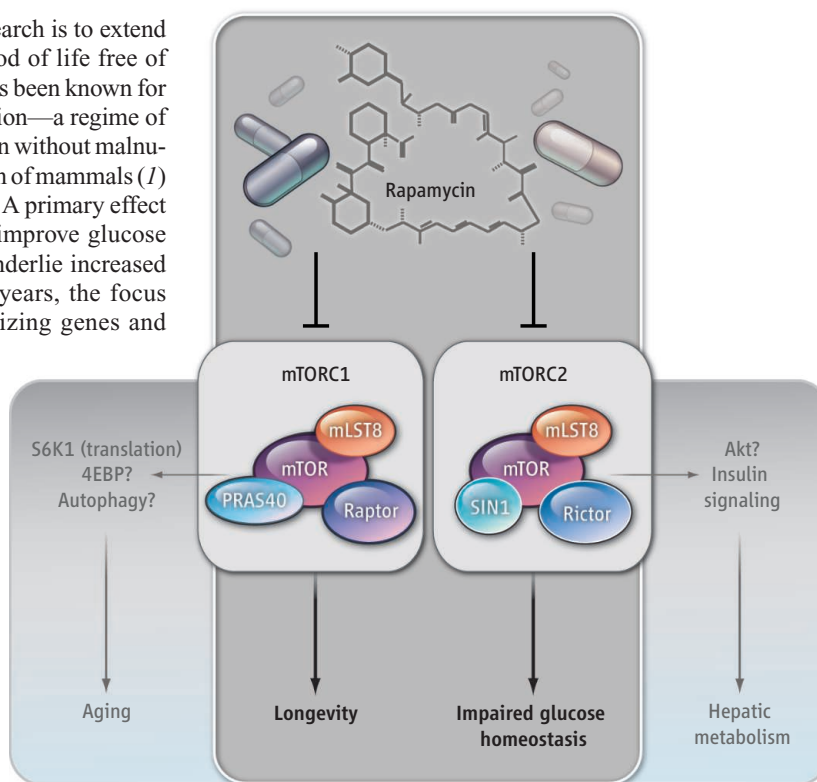
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Rapamycin Paradox Resolved

Katherine J. Hughes¹ and Brian K. Kennedy^{1,2}

The goal of aging research is to extend healthspan, the period of life free of chronic disease. It has been known for decades that calorie restriction—a regime of reduced calorie consumption without malnutrition—extends the life span of mammals (1) and other model organisms. A primary effect of calorie restriction is to improve glucose homeostasis, which may underlie increased longevity. In the last few years, the focus has turned from characterizing genes and molecular mechanisms that drive aging (2) to small-molecule interventions, and in 2009, rapamycin was the first drug reported to extend the life span of mice—roughly 15% in females and 10% in males (3). Rapamycin has been proposed as a calorie restriction mimetic and, at least in mouse models, it is proving beneficial in preventing the onset of many age-related diseases (4). However, there is a fly in the ointment regarding its use (or that of rapalog derivatives) to extend healthspan. Rapamycin causes glucose intolerance and insulin resistance in mice and humans (5), effects that could outweigh longevity benefits. On page 1638 of this issue, Lamming *et al.* (6) identify a mechanism by which the drug confers insulin resistance, and show that rapamycin's effect on glucose homeostasis and longevity can be uncoupled.

Discovered more than 40 years ago, rapamycin is now used clinically for a range of conditions, including cancer. It is a non-competitive and specific inhibitor of the nutrient-responsive mammalian target of rapamycin (mTOR) kinase (7). The mTOR protein is found in two protein complexes: mTORC1, which regulates pathways involved in mRNA translation, autophagy, and other cellular processes; and mTORC2,



Uncoupled effects. Inhibition of mTORC1 by rapamycin promotes longevity, potentially by blocking protein translation (S6K1 inhibition); a role for other mTORC1 effectors is not yet known. Inhibition of mTORC2 by rapamycin causes insulin resistance and impairs glucose homeostasis potentially by blocking Akt, a major enzyme in glucose uptake and insulin responsiveness.

which regulates the cytoskeleton and insulin signaling. mTORC1 was originally identified as rapamycin sensitive, whereas mTORC2 was thought to be rapamycin insensitive. However, chronic rapamycin treatment inhibits mTORC2 in vitro and in mice (8). The longevity effects of rapamycin are thought to be mediated by blocking mTORC1 activity given many observations: the specificity of rapamycin; the extended life span of female mice lacking S6 kinase 1 (S6K1), a target of mTORC1 (9); and the role of mTORC1 in longevity in invertebrates (3, 10, 11). But interestingly, adult worms with reduced expression of the mTORC2 component Rictor live longer (12). Given this finding, the issue of which mTOR complex is linked to life span in mice has not been adequately resolved.

Using genetic mouse models to ablate mTORC1 or mTORC2 activity (by deleting *Raptor* for mTORC1 and *Rictor* for

A small molecule acts through separate mechanisms to exert very different effects—extend mammalian life span or impair glucose homeostasis.

specific tissues, Lamming *et al.* observed the effects of rapamycin to disassemble complexes (see the figure). This was warranted because chronic treatment, at concentrations associated with longevity and impaired glucose homeostasis, inhibited both mTORC1 and mTORC2 in multiple tissues. A key finding was that liver-specific ablation of *Raptor*, impaired hepatic glucose homeostasis and that these mice were not further affected by rapamycin treatment. This observation and further evidence strongly support a model whereby rapamycin confers impaired glucose homeostasis through adverse effects on hepatic gluconeogenesis in a manner mediated by mTORC2 inhibition.

Yet, longevity studies of Lamming *et al.* support previous data that reduced mTORC1 activity extends mammalian life span. To address this paradox, the authors generated mice with reduced functional mTORC1. They characterized compound heterozygote mice—animals expressing reduced amounts of mTOR and mLST8 (*mtor*^{+/-}*mlst8*^{+/-}) because mice completely lacking *mTOR*, *Raptor*, or *Rictor* are inviable (13). Both proteins are present in mTORC1 and mTORC2 complexes. However, Lamming *et al.* observed that tissues from *mtor*^{+/-}*mlst8*^{+/-} mice had more pronounced defects in mTORC1 function. The authors speculate that these divergent effects on the two mTOR complexes may derive from preferential recruitment of the two proteins to mTORC2 when protein amounts are limiting. Similar to S6K1-deficient mice or mice treated with rapamycin, female *mtor*^{+/-}*mlst8*^{+/-} are long-lived. These findings strongly suggest that reduced mTORC1 function enhances longevity in mice.

Other mouse models of reduced mTORC1 function, such as *Raptor*^{+/-} or *mtor*^{+/-}*Raptor*^{+/-}, were not long-lived, suggesting that the right amount of mTORC1 inhibition, or perhaps

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the right balance of mTORC1 to mTORC2 activity, must be attained for enhanced longevity. Also, the findings do not rule out a role for mTORC2 in aging. What Lamming *et al.* do achieve is a clear dissociation of impaired glucose homeostasis and enhanced longevity. Indeed, *mtor^{+/-} mlst8^{+/-}* mice have normal glucose homeostasis, indicating that delayed aging derives from some other consequence(s) of mTORC1 inhibition.

Considerable effort is being directed to identify new and better mTOR inhibitors. The results from Lamming *et al.* lead to some early predictions about the ability of

such compounds to modulate longevity. For instance, high-specificity inhibitors that target the active site of mTOR, such as PP242 and Torin, have recently been developed (14). Although blocking both mTORC1 and mTORC2 function may be ideal for oncology and other conditions, these inhibitors may be less effective than rapamycin for increasing longevity and preventing age-associated diseases. The study by Lamming *et al.* may point the way toward such therapeutic approaches.

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COMPUTER SCIENCE

How Smart Is Your Home?

Diane J. Cook

Individuals spend most of their time in their home or workplace; for many, these places are their sanctuaries. Over the course of the 20th century, technological advances have helped to enhance the comfort and shelter provided by our homes. Insights gained from capturing and modeling behavior in these places may be useful in making our environments more intelligent and responsive to our needs. Recent advances are bringing such “ambient intelligence” in the home closer to reality.

Since the miniaturization of microprocessors, computing power has been embedded in familiar objects such as home appliances and mobile devices; it is gradually pervading almost every level of society. Ambient intelligence extends the notion of computing to provide customized, automated support that is so gracefully integrated with our lives that it “disappears” (1). In the home, the idea is that computer software playing the role of an intelligent agent perceives the state of the physical environment and residents using sensors, reasons about this state using artificial intelligence techniques, and then takes actions to achieve specified goals, such as maximizing comfort of the residents, minimizing the consumption of resources, and maintaining the health and safety of the home and residents.

During perception, sensors embedded in the home generate readings while residents perform their daily routines (see the figure). The sensor readings are collected by a com-

puter network and stored in a database that the intelligent agent uses to generate useful knowledge such as patterns, predictions, and trends. On the basis of this information, the intelligent agent selects an action and stores this selection in the database. The action is transmitted through the network to the physical components that execute the command. The action changes the state of the environment, triggering a new perception/action cycle.

Filling a home with sensors and controlling devices by a computer is not only possible, but it is simple and commonly found in homes today. Sensors are available off-the-shelf that localize movement in the home, provide readings for ambient light and temperature levels, and monitor usage of doors, phones, water, and appliances. Tiny, inexpensive sensors can be attached to objects to not only register their presence but also record histories of recent interactions. Such smart objects can harvest their own energy, and recent standards facilitate vendor-independent plug-and-play sensor design and modeling (2).

Proliferation of sensors in the home results in large amounts of raw data that must be analyzed to extract relevant information. Most smart-home data from environmental sensors such as infrared motion sensors and magnetic door sensors can be processed with a small computer. Once data are gathered from wearable sensors and smart phones (largely accelerometers and gyroscopes; sometimes adding camera, microphone, and physiological data), the amount of data may get too large to handle

Technical advances are bringing intelligent homes that respond to residents' needs and wishes within reach.

on a single computer, and cloud computing is appropriate. Cloud computing is also useful if data are collected for an entire community of smart homes to analyze community-wide trends and behaviors.

Currently, most users write rules by hand to interpret sensor data and to control devices. For example, home owners installing home automation equipment must write their own rules for when their lights turn on and off. Artificial intelligence (AI) plays a pivotal role in automating this process. AI and data-mining technologies seek useful information on resident behavior and the state of the home. Computer algorithms have been designed to predict and identify activities performed in the home and to recognize emotions, body mannerisms, and gestures (3, 4). These capabilities, as well as the abilities to recognize activities, identify trends, make assessments, and take action, are becoming more available and robust, but are not commonly found in actual homes.

The goal of much current research in ambient intelligence is to enable devices to interact with their peers and the networking infrastructure without explicit human control. The intelligent home must also be imbued with an awareness of the resident context (location, preferences, activities), physical context (lighting, temperature, house design), and time context (hour of day, day of week, season, year). Providing this type of context-aware reasoning makes it possible to design environments that provide, for example, customized lighting and temperature settings based on learned resident preferences; information retrieval and

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displays that follow residents from computer screen to wall to tabletop; and automated reminders to residents of important tasks they need to perform (5). As AI research progresses, these services will increasingly be provided on the basis of detected behavioral patterns, rather than rules provided by the user, as is currently the case.

Because ambient intelligence seeks to be both unobtrusive and ubiquitous, it can affect almost every aspect of human life without drawing attention to itself. Three applications of ambient intelligence in the home have received particular attention: health monitoring, energy efficiency, and social computing.

Ambient intelligent homes offer technologies for automated health monitoring, assessment, and intervention. For example, researchers have used capabilities found in ambient intelligent homes to find a link between changes in mobility patterns and the onset of symptoms of dementia (6). Motion sensors were placed throughout the home, and total space covered throughout the day as well as walking speed were estimated for individuals over multiple years. Changes in these parameters correlated with early symptoms of dementia. Other researchers have used ambient intelligence technologies to perform early-childhood screening for autism (7). Building upon context awareness, smart homes provide well-timed prompts to remind individuals to perform familiar routines and to initiate new health behaviors (8).

Monitoring energy consumption in the home is also increasingly important, because energy consumption is rising at a higher rate than population growth, and households are responsible for over 40% of total energy use in most countries. Ambient intelligent homes facilitate smart energy management through nonintrusive load identification and estimation, as well as minimally intrusive load shedding and automation for energy efficiency (9).

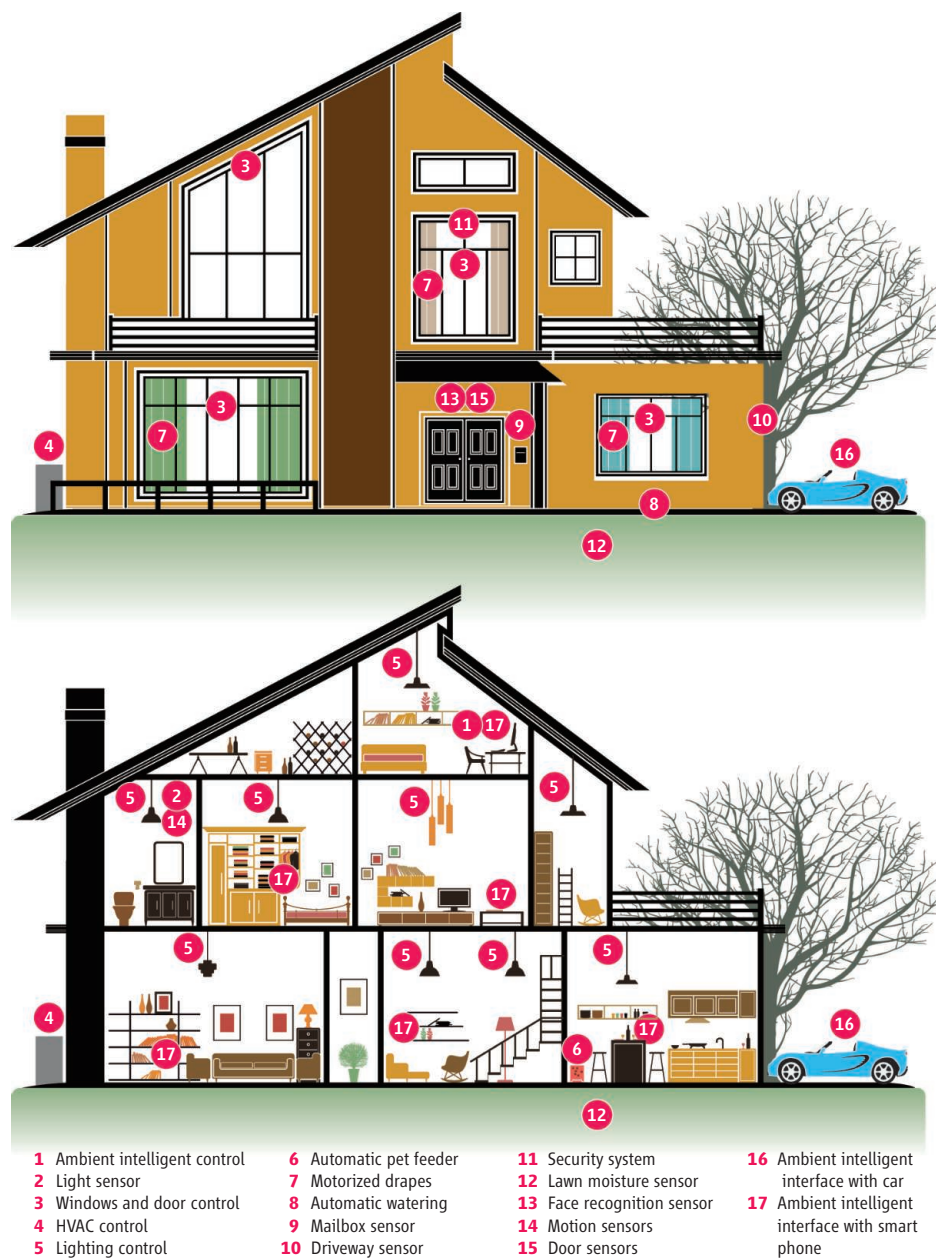
Another aspect of daily life is social interaction. Social signals have long been recognized as important for establishing relationships, but only with the introduction of sensed environments have researchers become able to monitor and measure these signals (10). Building upon ambient intelligence technologies, we can look at socialization within the home (such as entertaining guests, interacting with residents, or making phone calls) and examine the correlation between socialization parameters and productivity, behavioral patterns, and health. These results will help researchers

not just to understand social interactions but also to design products and behavioral interventions that promote interaction, or to leverage existing social relationships in ways that influence interventions and purchasing choices.

The dream of ambient intelligent homes is, however, hampered by some formidable challenges. A primary concern is the need to consider possible implications for privacy and security. Many individuals are reluctant to introduce sensing technologies into their home, wary of leaving digital trails that

others can monitor and use to their advantage, such as to break in when the house is empty. To allay such concerns, researchers are investigating ways to define and provide guarantees for levels of privacy and for the safety of the technologies. Similarly, smart homes need to ensure that the resident retains the ultimate authority to reset the system and to impose constraints that prevent the home from taking undesired or harmful actions.

Another critical challenge for home-based ambient intelligence is to provide



Coming soon to your home? In an ambient intelligent home, sensors collect information about the environment and the residents. An “intelligent agent” uses this information to decide whether actions need to be taken to adjust, e.g., temperature or lighting.

intelligent support seamlessly both inside and outside the home. With the introduction of wearable sensors and smart phones, ambient intelligence is not limited to the home environment (11). Our ambient intelligent agent needs to seamlessly merge support inside the home with that available in a mobile setting. Smart homes currently offer useful monitoring and automation with guidance from the resident. As the field matures,

we anticipate that these services will be provided in a more independent manner.

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COMPUTER SCIENCE

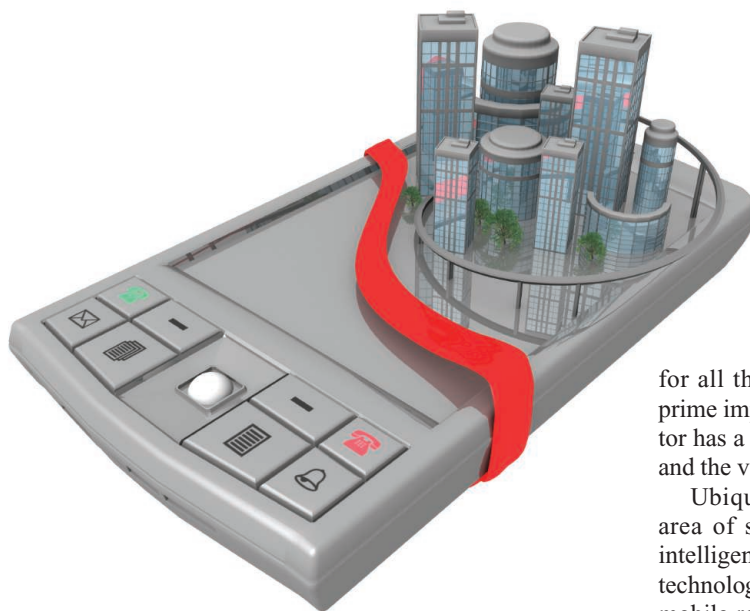
How Smart Is Your City?

Michael O'Grady and Gregory O'Hare

The idea of ambient intelligence implies an intrinsic link between individuals and their environment, enabling individuals to access and interact with computing artifacts in ways that are intuitive and do not disrupt everyday activities. Given the many different environments encountered as part of everyday life—within the home (1) as well as beyond it—enabling such interaction is a formidable technological challenge. The reward may be an environment that is safer, uses less energy, and responds to the needs of all individuals (see the figure). Recent advances in embedded systems, robotics, and sensor technology suggest that ambient intelligence may indeed be realized, particularly if crucial privacy and security concerns are addressed.

Three examples illustrate the potential of ambient intelligence across different domains. First, information filtering and personalization in a museum context offer a practical and eminently achievable vision of ambient intelligence. Second, autonomous mobile robotic services are a development that challenges perception, trust, and acceptance of ambient intelligence. Third, a simple ambient intelligence smart-phone service shows how the quality of life of those afflicted with dementia can be improved.

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From art galleries to our cities' streets, ambient intelligence can help to make the human environment more responsive to the needs of individuals.

A changing world. Through smart-phones and related devices, users have the world at their fingertips. But what if the city environment could respond automatically to individuals' needs and wishes?

ture—using radio-frequency identification (RFID), for example—and the construction of an information space for all the exhibits. This latter issue is of prime importance, demanding that the curator has a deep understanding of the exhibits and the visitors they hope to attract.

Ubiquitous robotics (3) represents an area of substantial potential for ambient intelligence in the longer term. Many of the technologies to successfully deploy suites of mobile robots exist. For example, the Dust-Bot project (4) has demonstrated robots collecting waste in an urban environment without direct human oversight, demanding obstacle (stationary and mobile) avoidance on the part of the robot, as well as interaction with householders. Computational intelligence, embedded both on the robot and in the environment, is essential for enabling this behavior. However, at present the cost remains excessive. Until this is addressed, robots cannot be deployed in a widespread fashion, and the key issues of human-robot interaction and social acceptance cannot be researched thoroughly.

Ultimately, ambient intelligence is about people and improving their quality of life. Consider the case of people with dementia, an issue that will have increasing implications for society in the coming years. Wandering is characteristic of many with dementia and is a major factor contributing to institutionaliza-

tion (5). Tracking is one solution, but raises ethical and privacy concerns (6). In equipping the person with a GPS unit—for example, embedded on a smart phone or a wearable wristwatch—models of their behavior can be constructed, deviations from which can lead to alarm generation. Crucially, the decision to activate an alarm can be made on the smart phone or wristwatch, thus removing the need for a centralized tracking approach. Hence, it is possible to balance the privacy of the person with dementia, their autonomy to make their own decisions, their own welfare, and the needs of their carers.

Proof-of-concept studies in these and many other domains, including offices, industry, transport, gaming, and e-commerce, demonstrate the potential of ambient intelligence. Indeed, increased awareness of energy issues suggests that the smart grid and energy efficiency (7) may become pioneering ambient intelligence services. Ultimately, researchers are envisioning “smart cities” (8) in which technologies such as ambient intelligence permeate all aspects of urban life. How can this potential be translated into a coherent vision and practical implementation, marked by scalability, robustness, and intelligence?

A key enabling technology is the Sensor Web (9), which envisages a network of sensors spatially distributed and embedded within the environment. However, a standardized approach is required to achieve interoperability between such physical sensor networks and the Web itself; in this way, seamless connectivity and usability will be achieved through a “Plug and Play” approach (10). The software required to achieve this, as well as being inherently distributed, must encapsulate robustness, scalability, and interoperability among others. Distributed Artificial Intelligence, specifically Intelligent Agents (11), is one mature software paradigm for delivering ambient intelligence.

A major constraint for the development of ambient intelligence systems will be how to power remote devices. Many embedded systems rely on battery or other finite power sources. Their life span may be weeks at most, frequently making ambient intelligence impractical. As the integration of low-power technologies into conventional consumer devices and ambient intelligence increases, energy harvesting from a variety of environmental sources will play a vital role in maintaining the long-term functioning of ambient intelligent environments (12). Sustainability or green computing precepts must also be factored into the design of ambient intelligence infrastructures. This

means that designers must plan for sustainable decommissioning, in much the same way that satellite engineers design satellites to burn on atmosphere reentry rather than being allowed to fall to Earth.

As ambient intelligence becomes mainstream, there is an urgent need for issues of security, privacy, and ethics to be brought to the public's attention. One interesting legal proposal is that of ambient law (13). This argues that the freedoms that underpin constitutional democracy may be radically altered as ambient intelligence permeates everyday life. Such freedoms may be reinforced in some circumstances; in others, they may be destroyed. Avoiding the latter situation demands greater communication between lawyers and computer scientists, such that appropriate legal norms can be constructed and incorporated into the design and use of ambient intelligence.

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NEUROSCIENCE

Segregation and Wiring in the Brain

Karl Zilles^{1,2} and Katrin Amunts^{2,3}

Are there unifying principles behind the structural complexity of the cerebral cortex?

A mosaic of hundreds of interconnected and microscopically identifiable areas in the human cerebral cortex controls cognition, perception, and behavior. Each area covers up to 40 cm² of the cortical surface and consists of up to 750 million nerve cells (1). The architecture—the spatial distribution, density, size, and shape of nerve cells and their processes—varies between different cortical areas. Nerve cells are interconnected within each area and with other brain regions and the spinal cord via fiber tracts, synapses, transmitters, modulators, and receptors. This incredible structural complexity underlies the functional segregation in the cerebral cortex. The ultimate goal—to understand the driving forces and organizational principles of the human brain beyond the cellular and functional details—remains a challenge. Reports by Chen *et al.* (2) and Wedeen *et al.* (3) on pages 1634 and 1628 of

this issue, respectively, accept this challenge by analyzing the genetic topography of the cortex and the spatial course of fiber pathways in the brain. The studies find unifying hierarchical and geometric rules behind the organizational details.

In addition to classical cytoarchitectonics (the spatial organization of cellular composition) of the brain (4), the fields of cell biology, neuroimaging, neuroinformatics, and genetics have provided myriads of valuable data about the structure and function of the cerebral cortex. Only after the advent of modern genetics was it possible to identify the driving forces behind segmentation as an organizational principle of the vertebrate brain. However, a segmentation process in the cortex, similar to that seen in other brain regions and the spinal cord, does not seem likely because an overt segmental or metameric partitioning of the neocortex is not visible during embryogenesis (5). Instead, Chen *et al.* demonstrate a genetically controlled and hierarchically organized structural segregation in the human cortex.

Chen *et al.* investigated human brains by cortical surface reconstruction, advanced

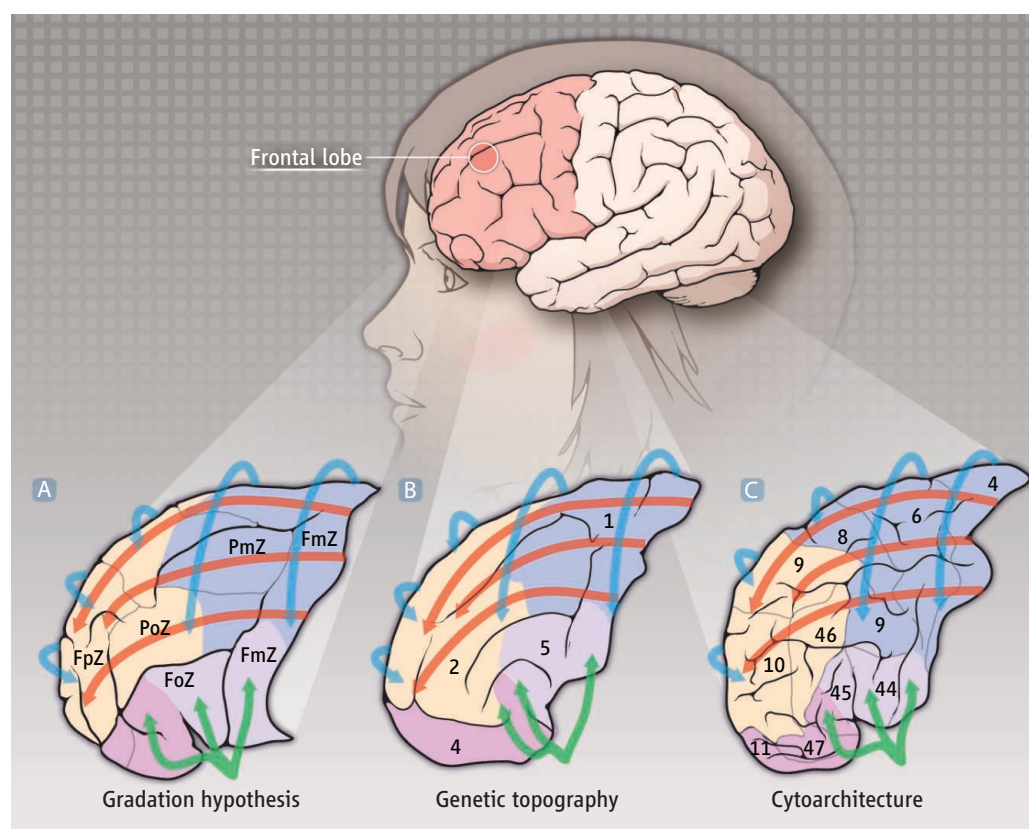
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atlas mapping, and genetic analyses. By comparing mono- and dizygotic twins, the authors calculated how shared genetics influence area expansion between cortical regions. A cluster analysis of this genetic topography was performed in the absence of predefined anatomical information. The authors could thus parcel the cortical surface area into 12 genetic regions. A possible correlation between this genetic topography and the cytoarchitecture of the cortex (6) may reveal rules behind the mosaic of cortical area. However, some genetically determined regions are very large, even crossing the borders of cytoarchitectonic areas and expanding into other lobes. For example, the occipital and inferior parietal clusters of Chen *et al.* comprise numerous cytoarchitectonic areas (7, 8).

Although there is mismatch in some details, this should not obscure an important commonality revealed between cytoarchitecture and genetic topography—hierarchical organization. Cytoarchitectonic maps reflect functional and molecular aspects of brain organization. The regional distributions of transmitter receptors (9), cell types (10), and connectivity (8), as well as the genetic topography determined by Chen *et al.*, are hierarchically organized. That is, cortical subdivisions are nested in a common superstructure and interrelated in a tree-like organization. They show different degrees of functional, molecular, or structural similarities. Hierarchical organization of the cortex is thus the unifying rule, which encompasses all scales from the molecular to the systems level.

The concept of hierarchy is reminiscent of an old but unfortunately forgotten finding (11–13): the gradation of architectonic features. This concept explains differences between cortical areas by a stepwise and directed architectonic differentiation of the neocortex. These developmental gradation streams follow non-Euclidean axes. The rostrocaudal axis is bent around the end of the lateral fissure; the other axes are orthogonal to it. In the frontal lobe (see the figure), gradation streams originate in the border regions of the insula, anterior cingulate gyrus, olfac-



Genetic topography, gradation, and cytoarchitecture. Regional organization of the human frontal lobe is shown according to the gradation hypothesis (A), genetic topography (B), and cytoarchitecture (C). Corresponding regions are highlighted with colors. Dark black lines represent sulci; thin gray lines mark boundaries of the zones. Blue, red, and green arrows label the architectonic gradation streams from the cingulate cortex, the central sulcus, and the insular cortex, respectively. Regions in (A): FmZ, frontomotoric zone; FoZ, fronto-opercular zone; FpZ, frontopolar zone; PmZ, paramotoric zone; PoZ, paraopercular zone. Regions in (B): 1, motor-premotor cluster; 2, dorsolateral-prefrontal cluster; 4, orbitofrontal cluster; 5, opercular-subcentral cluster. In (C), numerals indicate Brodmann areas (6) (regions defined according to the structure and organization of cells).

tory area, and central sulcus (13). These regions are phylogenetically old or are located in primary sulci (depressions or fissures in the surface of the brain). Along the gradation streams, the cellular composition and organization change stepwise according to the changing relationship between the sizes of layer V to layer III pyramidal cells in the cortex, along with the development of an inner granular cell layer IV. Thus, the gradation hypothesis describes a hierarchical rule behind the details of architectonic phenotypes. The results of Chen *et al.* suggest that the gradation topography principally corresponds to the genetic topography.

Previous studies of fiber tracts—bundles of nerve fibers—provided a wealth of detail about their spatial orientation in a highly descriptive, but not analytical way. Wedeen *et al.* propose a rectilinear, grid-like geometric organization of fiber pathways in human and nonhuman primate brains. Starting from major paths, the authors identified adjacent paths that cross the initial ones orthogonally.

The crossings form well-defined curved, two-dimensional sheets. The major longitudinal paths provide the long-range anatomical connectivity. The short U-fibers connect adjacent gyri (ridges on the cerebral cortex). As in the case of gradation streams, the three-dimensional grid of fiber tracts follows the three principal axes of growth direction during brain development (longitudinal, mediolateral, and dorsoventral). This organization may be achieved by chemotactic mechanisms of neuronal path finding and incremental rewiring.

Wedeen *et al.* also used the Frobenius theorem from differential geometry to support their argument for the formation of geometric spatial organization of fiber paths. At present it is difficult to decide whether three chemotactic gradients are the basis for the three families of vector fields observed by the authors, and whether other constraints of this theorem are fulfilled. If this “three-families hypothesis” as geometric principle holds true, procedures to map neural fiber

tracts can be constrained, which might make it easier to avoid the limitations of current algorithms and to validate different tracking techniques (across scales, across subjects, across species, etc.).

A common view that arises from the studies of both Chen *et al.* and Wedeen *et al.* is the idea of the brain as a regionally highly differentiated, but hierarchically and geometrically organized, spatial structure. Detailed aspects of this “canonical brain organization” can be modified by environmental conditions including pathology and genetic diversity. Mathematical methods such as hierarchical

clustering and differential geometry can help us to understand the principles behind variable phenotypes and to guide the development of a realistic brain model.

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PHYSICS

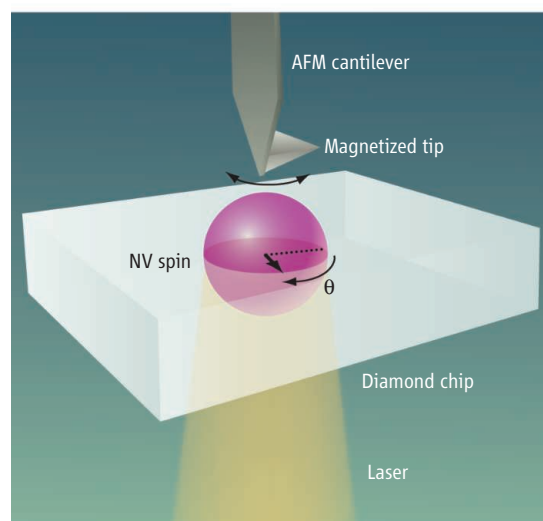
A Single Spin Feels the Vibrations

Philipp Treutlein

Mechanical resonators find widespread applications as precision force sensors, the most prominent example being the atomic force microscope (AFM). Coupling the vibrations of a mechanical resonator to a fully controlled, microscopic quantum system such as a single spin presents a strategy for detecting and even controlling mechanical vibrations in the quantum regime. The resulting hybrid quantum system would offer new perspectives for precision force sensing and tests of quantum mechanics on a macroscopic scale. On page 1603 of this issue, Kolkowitz *et al.* (1) have taken a first step toward such coupled spin-resonator systems by using a single electronic spin to sense mechanical vibrations of an AFM cantilever with a magnetic tip.

Observing and manipulating quantum behavior of mechanical objects is a goal currently being pursued through several different experimental approaches (2). Although quantum-level control over mechanical vibrations is routinely achieved for atomic-scale objects such as trapped ultracold atoms and ions (3), achieving a similar level of control over microstructured mechanical resonators such as cantilevers, beams, and membranes is far more challenging. Observing quantum behavior with a mechanical resonator that is visible to the naked eye, such as an AFM cantilever, would not only be a beautiful confirmation of quantum theory but may also lead to novel applications in precision force sensing (4).

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Spin flips in sync with vibrations. A single spin in a diamond crystal can be used to read out the vibrations of an AFM cantilever. The magnetized cantilever tip induces a coupling between the cantilever vibrations and the spin resulting in a spin rotation by an angle θ . The quantum state of the spin is subsequently detected with a laser.

is desirable to achieve similar control in other systems with longer coherence lifetimes. Moreover, from a standpoint of applications in force sensing, it is important to achieve quantum-level control over the fundamental center-of-mass vibration of a cantilever resonator, as used in an AFM. A number of different qubit-cantilever systems are currently being investigated for this purpose, involving systems from solid-state physics (6) as well as ultracold atoms and ions (7).

In order to observe quantum behavior, the resonator must be cooled to sufficiently low temperatures to avoid thermally excited vibrations. Equally important, tools are needed to read out the resonator and to determine and possibly even control its quantum state. One strategy is to couple the mechanical resonator to a microscopic quantum system, ideally a single two-level system (a “qubit”) that can be fully controlled quantum mechanically and can be read out efficiently. In a recent landmark experiment (5), a superconducting phase qubit was coupled to an internal mechanical vibration of a piezoelectric resonator at millikelvin temperatures and used for preparation and detection of nonclassical quantum states. The coherence lifetimes of the qubit and resonator quantum states were in the nanosecond range, and it

The spin of a single nitrogen vacancy (NV) center in diamond—the qubit system used by Kolkowitz *et al.*—is very promising. Its quantum state can be initialized and read out optically, it can be manipulated by applying microwave radiation, and it shows remarkably long coherence times—up to a few milliseconds even at room temperature (8). The spin can be coupled to the vibrations of a cantilever (9) by attaching a tiny magnet to the cantilever tip and positioning it near the diamond surface (see the figure). The strong magnetic field gradient that is produced translates the vibrations of the cantilever into an oscillating magnetic field, which couples

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to the NV spin via its magnetic moment. The resulting change of the spin's quantum state can be detected optically.

The interaction of a single spin and a mechanical resonator has already been studied from a somewhat different perspective: Using an ultrasensitive mechanical cantilever with a magnetic tip, researchers detected single electron spins embedded in a solid material (10), a technique known as magnetic resonance force microscopy. The experiment of Kolkowitz *et al.* turns this situation around and uses the spin to sense mechanical vibrations. During the measurement, the quantum state of the spin is controlled by microwave radiation. A clever sequence of coherent manipulation pulses is applied to the spin in order to enhance its sensitivity to the vibrations while suppressing noise from other sources. In this way, Kolkowitz *et al.* sense mechanical vibrations down to a few picometers in amplitude.

In a previous experiment on spin-resonator coupling, Arcizet *et al.* coupled an NV spin to the vibrations of a SiC nanowire (11). The NV was fixed to the tip of the nanowire while the magnet was placed near to it. They observed nanowire vibrations of a few tens of nanometers in amplitude through a change in the line shape of the NV spin resonance. Kolkowitz *et al.* used the coherent quantum

dynamics of the NV spin to reach higher sensitivity to mechanical vibrations.

The resonator motion detected here is in the classical regime at room temperature, but with the magnetic coupling mechanism, it is in principle possible to reach the quantum regime (6). Several technological improvements are necessary to reach this goal, including fabrication of a smaller cantilever with a much higher mechanical quality factor and a magnet made from a different material, and working at cryogenic temperatures. Ultimately, the system will have to reach the so-called strong-coupling regime, where the excitation of a single quantum of vibration (a single phonon) in the resonator is sufficient to flip the spin, and a single spin flip is sufficient to excite vibrations of the resonator. In this regime, the system realizes a mechanical analog of current experiments in cavity quantum electrodynamics (12), where the internal state of a single atom is strongly coupled to a single quantum of the electromagnetic field (a photon) inside an optical cavity.

A strongly coupled spin-resonator system in the quantum regime bears some analogy with the infamous Schrödinger's cat: The microscopic spin could be prepared in a quantum mechanical superposition state of pointing up and down at the same time. It could then influence the macroscopic mechani-

cal cantilever (playing the role of the cat) so that the cantilever ends up in a superposition state of vibrating in two opposite directions at once. Although some caution is in order—the cantilever contains a macroscopic number of atoms, but a superposition state involving only a few phonons is still in some sense microscopic—such an experiment would certainly be intriguing. From an applied perspective, the use of a single spin, the most elementary quantum system, to read out mechanical vibrations opens the path to force detectors operating at the ultimate limits of sensitivity. The experiment of Kolkowitz *et al.* is an exciting first step in this direction.

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GEOCHEMISTRY

Keeping Time with Earth's Heaviest Element

Claudine H. Stirling

Uranium is the heaviest naturally occurring element on Earth. It has three natural isotopes (^{238}U , ^{235}U , and ^{234}U), of which ^{238}U and ^{235}U are the parent nuclides of the ^{238}U - and ^{235}U -decay series chains, which ultimately decay to stable isotopes of lead (Pb), thereby forming the basis of the U-Pb chronometer. Conventional theories of stable isotope fractionation have dictated that uranium is too heavy to display resolvable mass-dependent isotope effects. The expectation was that Earth would display homogeneous $^{238}\text{U}/^{235}\text{U}$ isotopic compositions. The convention has been

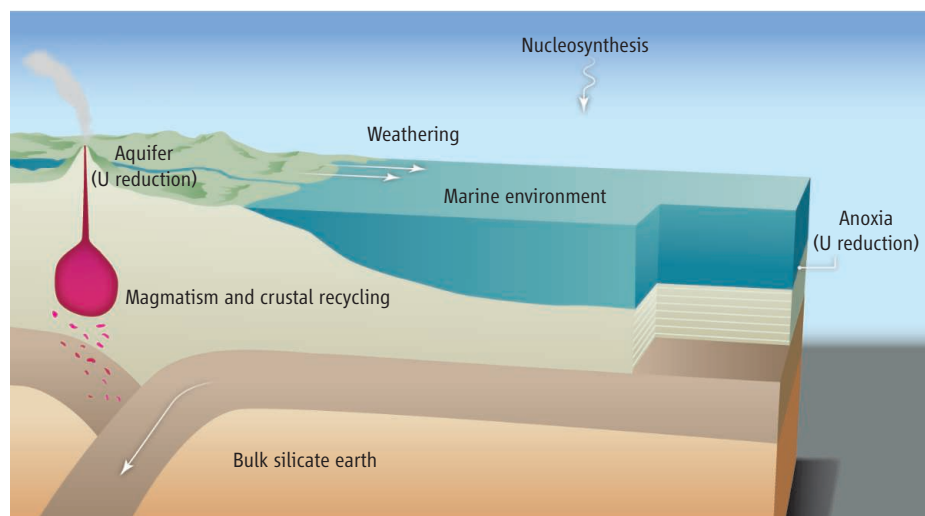
to adopt an invariant present-day $^{238}\text{U}/^{235}\text{U}$ ratio equal to 137.88 throughout the solar system, on the basis of early studies of uranium ore deposits. This critical assumption, which underpinned the veracity of the U-Pb chronometer for the past 30 years, was overturned by the discovery of surprisingly large $^{238}\text{U}/^{235}\text{U}$ variations in Earth's surface environments (1, 2). On page 1610 of this issue, Hiess *et al.* (3) report the $^{238}\text{U}/^{235}\text{U}$ composition of a large suite of U-bearing accessory minerals to facilitate a more accurate U-Pb geochronometer. These new results also provide fundamental but unexpected insights into the mechanisms controlling $^{238}\text{U}/^{235}\text{U}$ fractionation.

Together with the other actinides, uranium was produced over the lifetime of the

New $^{238}\text{U}/^{235}\text{U}$ ratios for uranium-bearing minerals provide a better chronometer for dating geological processes.

galaxy in massive exploding stars (supernovae) and was then incorporated into Earth during the formation of the solar system more than 4.5 billion years ago. In the past decade, high-precision measurements of $^{238}\text{U}/^{235}\text{U}$ were initially driven by the search for the former existence of extinct ^{247}Cm (4, 5), the short-lived precursor nuclide of ^{235}U . ^{247}Cm is a crucial actinide for evaluating models of solar system formation and is detected as small positive anomalies in the $^{238}\text{U}/^{235}\text{U}$ ratio of extraterrestrial meteorites (6). Contrary to expectations, initial measurements of $^{238}\text{U}/^{235}\text{U}$ in terrestrial reference materials revealed surprisingly large isotopic variability of approximately 1 per mil (‰) in a wide range of low-temperature environments (1, 2). These isotopic shifts

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Uranium $^{238}\text{U}/^{235}\text{U}$ cycling in the environment. Most terrestrial and extraterrestrial materials formed in high-temperature environments define the $^{238}\text{U}/^{235}\text{U}$ composition of “unfractionated” bulk silicate Earth. Superimposed on bulk silicate Earth are natural variations in $^{238}\text{U}/^{235}\text{U}$ in some other environments: Percent-level excesses in ^{235}U and low $^{238}\text{U}/^{235}\text{U}$ values are found in some mineral inclusions of extraterrestrial meteorites; in the hydrological environment, uranium reduction leads to “light” $^{238}\text{U}/^{235}\text{U}$ signatures in the unreacted U(VI) water and “heavy” compositions in aquifer deposits and organic-rich seafloor sediments containing reduced U(IV); leaching of exposed minerals during chemical weathering results in marginally low $^{238}\text{U}/^{235}\text{U}$ values in the modern marine environment (including seawater precipitates). Some materials fractionated in these and other low-temperature processes are recycled at depth through crustal recycling. Additional $^{238}\text{U}/^{235}\text{U}$ fractionation likely operates at magmatic temperatures.

cannot be explained by conventional theories of stable isotope fractionation. Instead, they appear to be largely controlled by the mass-independent nuclear field shift effect (7, 8), which arises from varying nuclear volumes and electron density distributions between different isotopes of the same element. Isotopic fractionation governed by the nuclear field shift favors heavy isotopes in the lower oxidation state where the electron density near the nucleus is lower, and is predicted to have a strong effect on the heavy masses.

Natural variability in $^{238}\text{U}/^{235}\text{U}$ will undoubtedly lead to revision of some critically important cosmochronological and geochronological models. Coupled measurements of $^{238}\text{U}/^{235}\text{U}$ and $^{207}\text{Pb}/^{206}\text{Pb}$ have already begun in the field of cosmochronology, where models of solar system formation are often based on relative age differences of only a few million years (9). Hiess *et al.* have initiated efforts in the terrestrial realm by characterizing the $^{238}\text{U}/^{235}\text{U}$ composition of a diverse range of U-bearing minerals that are essential in U-Pb geochronology. These new data display extremely large $^{238}\text{U}/^{235}\text{U}$ isotopic shifts exceeding 5‰. The most extreme compositions are displayed by titanite and monazite, and more uniform $^{238}\text{U}/^{235}\text{U}$ signatures are observed in zircon. The implication is that previous U-Pb ages for these accessory minerals may be inaccurate by up to 10

million years. Hiess *et al.*'s study reemphasizes the need to independently characterize the $^{238}\text{U}/^{235}\text{U}$ composition of every sample to obtain accurate U-Pb chronologies. Because this new data set is traceable to the International System (SI) of units, it can facilitate a revised, albeit provisional, estimate of the decay constant for ^{235}U .

Efforts in the field of uranium “stable” isotope geochemistry have also focused on gaining an improved understanding of the mechanisms controlling $^{238}\text{U}/^{235}\text{U}$ fractionation in low-temperature environments where the largest isotopic shifts have occurred. In this context, the U(VI)-U(IV) redox transformation has received considerable attention (1, 2, 10–13). Specifically, the reduction of uranium in water results in a dramatic removal of dissolved U. In parallel, a corresponding isotopic shift toward depleted $^{238}\text{U}/^{235}\text{U}$ signatures occurs in the unreacted U(VI) water because of the nuclear field shift effect. Therefore, the ^{238}U - ^{235}U system already demonstrates considerable promise as a tool to monitor redox conditions in U bioremediation studies (11) and as a paleo-redox tracer to quantify the extent of anoxia in the historical oceans (14).

Hiess *et al.* extend our understanding of the mechanisms controlling $^{238}\text{U}/^{235}\text{U}$ fractionation for several reasons. First, the reported data set unequivocally shows that $^{238}\text{U}/^{235}\text{U}$ variability is not limited to low-

temperature settings but is also prevalent in higher-temperature magmatic environments, and strongly implies that material fractionated through low-temperature processes is incorporated into higher-temperature systems through crustal recycling (see the figure). Second, the magnitudes of $^{238}\text{U}/^{235}\text{U}$ isotopic shifts in high-temperature magmatic environments appear to rival those in lower-temperature systems. Third, their compilation of all previously published $^{238}\text{U}/^{235}\text{U}$ observations to provide a global, SI-traceable data set allows for a rigorous comparison among laboratories and reveals the true complexity of the ^{238}U - ^{235}U system. The majority of samples show a relatively uniform $^{238}\text{U}/^{235}\text{U}$ composition that gives rise to an absolute $^{238}\text{U}/^{235}\text{U}$ value of 137.818 ± 50 for “bulk silicate Earth.” However, there is a 0.4‰ enrichment in ^{235}U over ^{238}U in the present marine environment that appears to be driven by the long-term chemical weathering of exposed crustal rocks and preferential release of lattice-bound ^{235}U from the leaching of exposed minerals. This surprising result corroborates earlier findings (1) and gives rise to a correlation between the relative abundance of ^{235}U and ^{234}U in some low-temperature environments, even though the excess ^{234}U is not lattice-bound and need not be correlated with ^{235}U release at all. The study by Hiess *et al.* therefore supports the idea that $^{238}\text{U}/^{235}\text{U}$ variability is not exclusively controlled by the U(VI)-U(IV) redox transformation and will undoubtedly drive future directions in uranium “stable” isotope geochemistry.

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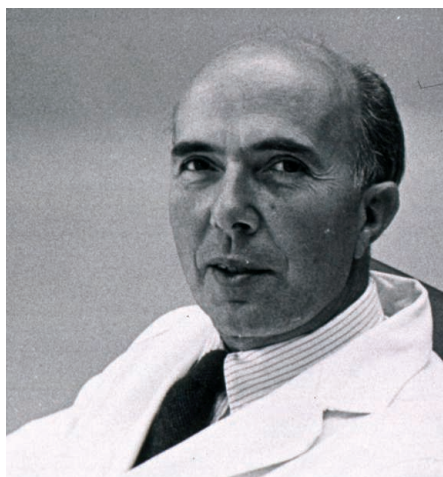
Renato Dulbecco (1914–2012)

David Baltimore

When a gentle superman passes from our midst, we must bow our heads in recognition of his powers. Renato Dulbecco was both gentle and remarkable. He died on 19 February at his home in La Jolla, California. I got to know Renato when he invited me in 1965 to set up my first laboratory within his space at the then-nascent Salk Institute. He was moving from a professorship at the California Institute of Technology (Caltech), where he already had a notable career in virology. We were to share a Nobel Prize, with Howard Temin, just 10 years later.

Renato was born in 1914 in Catanzaro, Italy. He was a student in the memorable pre–World War II laboratory of Giuseppe Levi at the University of Turin, along with two other Italians who, like him, came to America and won Nobel Prizes—Rita Levi-Montalcini and Salvador Luria. He became a physician, was conscripted into the Italian army to serve on the Russian front, defected, and became a partisan. Luria meanwhile had gone to France and then the United States. While at Indiana University he asked Renato to join him in Bloomington. In 1947, Renato went to Indiana as a research associate and began his great career in life science research.

He began working on aspects of bacteriophage genetics, notably discovering the process of photoreactivation of ultraviolet-irradiated bacteriophage. After 2 years, he joined the faculty at Caltech, where he continued his phage work. But a donor had given Max Delbrück money to work on animal viruses, a challenge picked up by Renato because he recognized it as a wide-open field with particular relevance to medicine. Within a year, in a single-author paper, he described the solution of a key problem holding back animal virology—the lack of a quantitative assay to enumerate live virus particles. Renato then teamed up with Marguerite Vogt, another European refugee who had come to Caltech to breathe the fresh air of American science. As his life-long scientific associate, they together used quantitative methods to understand many aspects of viral growth.



In the late 1950s, influenced by his student Howard Temin and his postdoctoral fellow Harry Rubin, who were working on Rous sarcoma virus, Renato shifted his interest to the newly discovered polyoma virus, a tumor-producing virus. He focused on understanding cell transformation—the *in vitro* analog of cancer induction—becoming particularly interested in the ability of the virus to stimulate cellular DNA synthesis. In the early 1960s, as Renato's interest in cancer blossomed, he also became intrigued by the plans of Jonas Salk to set up an institute in La Jolla, California, where he would be free of the usual academic constraints. At Salk, I joined Renato for almost 3 years, where we worked in temporary wooden buildings (still standing) until the grand Louis Kahn edifice was finished. Renato was keen to investigate polyoma-induced cell transformation, leading him to spend time with Michael Stoker in Glasgow before a planned return to the Salk Institute. From early on at Salk, Renato recognized that the DNA of the virus was the active agent causing cell transformation. In 1968, he and Joseph Sambrook showed that the viral DNA was integrated into the cellular DNA and proposed that the virus was adding genes to cells, implying that genes cause cancer. This paper capped his involvement in the study of virus-induced cancer and won him the Nobel Prize in 1975.

Howard Temin and I shared that Nobel Prize, and although we both worked side by side with Renato and were influenced by his clarity of thought on very difficult problems, neither of us ever published with him. Many

A Nobel Prize-winning virologist discovered a connection between viruses, genetic mutations, and cancer.

who worked with Renato went on to lustrous careers, notably Lee Hartwell, Robert Weinberg, and Joseph Sambrook.

After receiving the Nobel Prize, Renato decided to focus on human breast cancer rather than staying with the somewhat artificial problem of virus-induced cancer. He continued this interest until his death, publishing as late as 2008 (when he was 94) with a group that he maintained in Milan, Italy, in his later years.

The clarity of Renato's understanding of the cancer problem is probably best underlined by a Perspective he wrote for *Science* in 1986 entitled "A Turning Point in Cancer Research: Sequencing the Human Genome." Noting the daunting genetic complexity of cancers, he said, "We have two options: either to try to discover the genes important in malignancy by a piecemeal approach, or to sequence the whole genome..." He strongly argued for the second option, calling for an "international undertaking" and recognizing that it would have utility far beyond the cancer problem. It took many years of effort for his dream of sequencing the human genome to come to reality, but there is no question who initiated the process.

Renato was a gracious man, but quite formal in his demeanor. He loved music, enjoyed golf, was a widely read intellectual, and cared deeply about science. As I knew him in his later years with a second family, he was a deeply caring family man, raising a wonderful daughter. Much credit must go to his wife Maureen, who was perhaps more effectively grounded in day-to-day reality and often seemed to guide Renato through it.

Renato lived through the revolution we call molecular biology from its inception, leading its application to animal viruses, mammalian cell biology, and cancer. Interestingly, the other person who led that development was his mentor, Salvador Luria, and I count both of them as my guides. There are few people left who participated in the birth of molecular biology, but the imprint of this band of innovators on our understanding of the nature of life is indelible. Renato was one who played multiple roles, every time seeing over the horizon to what was possible and finding ways to bring us to that new level of understanding.

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IBI* SERIES WINNER

Investigating Arabia Mountain: A Molecular Approach

Nitya P. Jacob

Exposing undergraduate students to inquiry and discovery, elements that lie at the core of science, is an effective strategy to lure them into the scientific world. This is typically accomplished when individual students partner in research with a faculty member on an independent project. However, such opportunities to participate in research are limited to a small number of students in their third and fourth years of college. Ideally, all students should begin their journey into the world of scientific discovery as early as possible.

Teaching at Oxford College, an undergraduate division of Emory University focused on the liberal arts education of first- and second-year students, gave me the inspiration to lead such a journey. I designed a research module, *Investigating Microbial Diversity on Arabia Mountain: A Molecular Approach*, for the laboratory component of Biology 142: Advanced Topics in Genetics and Molecular Biology, the second course in our introductory biology curriculum. The first course, Biology 141: Cell Biology and Genetics, establishes basic foundations of research in the laboratory: learning general techniques, repeatedly applying the scientific process, researching literature, and practicing written and oral scientific communication, through a variety of exercises (1). Taking students to a new level of being actively involved in an authentic research experience in the second course became the natural next step.

A unique feature of the local Georgia landscape is the presence of several exposed granite rock outcrops, the most famous being Stone Mountain. Plant communities, ecological succession, and evolution of these granite outcrops have been studied in detail over many years, by a series of Emory University biologists (2, 3). However, not much is known about the microbial ecology in this ecosystem. Exploring the extent of microbial life on the planet has recently come to the forefront of biology (4). The



Arabia Mountain, Lithonia, Georgia. Students collect samples from a variety of environmental niches dispersed in depressions on the exposed rock outcrop surface.

topic of investigating microbial diversity on Arabia Mountain, a granite rock outcrop near Oxford, Georgia, provides an opportunity to use the tools of molecular biology in an ecological setting, an approach used in current studies (5) (see the photos).

The 11-week laboratory research module has four components: thinking time, field experience, laboratory bench work, and communication. Thinking time is the most essential element of this module. In a class of 24 students, six research teams of three to four individuals each design an original research question under the broad umbrella of investigating microbial diversity in the environment of a granite rock outcrop. Students research existing literature on microbial and plant ecology during a scheduled laboratory period. The instructor and a librarian check in periodically with each group to provide guidance as needed. Linking this module with a long-standing collaboration between the Biology department and the library was central to achieving information literacy learning goals throughout the project (6). After consulting scientific literature and active collaborative discussion in the classroom, each research team writes a proposal presenting a specific research question and a corresponding hypothesis with a well-substantiated rationale. The research questions posed by each group are shared with the entire class and the instructor helps teams to establish a common connection between individual questions, enabling each team to later analyze their data in the context of discoveries made by other teams in

"Investigating Microbial Diversity on Arabia Mountain," the IBI Prize-winning module, allows students to explore microbial diversity by DNA isolation and sequencing.



the same class. The laboratory module continues with field experience at Arabia Mountain where soil samples are collected onto nutrient agar plates from various locations according to specific research questions.

In the 6 weeks that follow, research teams combine laboratory benchwork and thoughtful analysis to select morphologically distinct bacteria from their collections, amplify 16S ribosomal DNA (rDNA) from genomic DNA by the polymerase chain reaction, conduct restriction fragment length polymorphism analysis, and analyze 16S rDNA sequences with software tools (sequencing is completed at an off-campus facility)—all techniques that are new to them in this course (7, 8). Thinking time continues in weeks 7 to 9 of the module when students examine and synthesize their data by actively discussing outcomes and arguments, with easy access to the instructor as a resource. In data synthesis, each student team also examines data generated by other teams, shared on the course's Blackboard Web site, to make final conclusions about their results (see the photo, page 1589).

Finally, in the communication component of the module, each team of students presents their project to the class in a research seminar format. Additionally, each student writes an individual paper on the project in scientific manuscript style.

The creative process of designing the question and later thoroughly analyzing the data as a group and as an individual is completely owned by the students. There is less flexibility in the experimental design of the

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laboratory procedure for practical reasons, such as the level of student experience and laboratory preparation constraints for an introductory course. Results generated by student projects provide preliminary findings on microbial community structure in outcrop soils. Projects have then been continued as independent research by students who have presented posters at summer research and regional society meetings and whose work is being prepared for publication. Another valuable outcome is increased interest and participation of our students in summer undergraduate research programs nationwide.

Understanding and testing the feasibility of the project with first- and second-year students required adjustments over several semesters since launching this module for the first time in 2006. A major challenge for students is the frustration and uncertainty that they are faced with at various points in the project.

Frustration begins in the very first week of the module. There are no existing publications specifically on the microbial diversity of granite rock outcrops in Georgia, and students expect to find direct answers in the literature while constructing a hypothesis. For example, connecting information from published studies on soil bacteria to propose a hypothesis about bacteria living in outcrop soils is not an easy task for them. While analyzing data, students struggle with making sense of information. They often find unexpected results, not consistent with their hypothesis, such as a lower level of bacterial diversity than expected or finding bacteria with variable morphological forms identified as the same species by DNA sequence analysis. Synthesizing the data of other teams for a comparative anal-

ysis in their report is also a difficult exercise. Overcoming these challenges requires the instructor and students to work together as true partners in scientific discovery. The instructor should be prepared not to have all the answers for the students, but instead should ask the right questions to help them move in a positive direction.

The rewards of getting through this challenging process come at the end of the semester. Students learn that research is not straightforward and outcomes are often unexpected. When pushed into an uncomfortable space with unexpected research results, students learn to think deeper to explain problems. Their final papers often show a thorough survey of scientific literature on the topic, creative presentation of data, and thoughtful arguments to explain results. Achieving this final point requires targeted guidance and engagement on the part of both student and instructor.

What has mattered most in this module is the learning process itself. The actual topic of research has been secondary to the process, although a project with a connection to the local environment has been meaningful for the students. Going through each step of the scientific discovery process, including its uncertainty and frustration, gives students a realistic view of thinking and working like a scientist. Working collaboratively with peers in laboratory procedures—in making decisions about data analysis and presenta-

About the author



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tion—and in actively discussing arguments stresses the critical role of collaboration in scientific discovery and inquiry. Previous preparation and experience with scientific investigation and inquiry in the first course ensured more success for students (9).

The open-ended nature of investigating outcrops allows the research module to evolve from year to year, exploring new approaches and questions informed by past student projects. Each new class thus remains enthusiastic about being part of a larger research initiative. Expanding the framework of this module to incorporate new topics or interdisciplinary connections and teaching students how to effectively use shared data are future goals.

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Supporting Online Material

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Thinking time! Students work collaboratively in research teams to actively discuss data analysis and synthesis.

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Adapting to Osmotic Stress and the Process of Science

Brittany J. Gasper,^{1†} Dennis J. Minchella,¹ Gabriela C. Weaver,^{2,3} Laszlo N. Csonka,¹ Stephanie M. Gardner^{1‡}

"Genetic Analysis of Adaptation to Osmotic Stress in *Salmonella*," the IBI Prize-winning module, uses mutant isolation and DNA sequencing to engage students in bacterial genetics and evolution.

Inquiry-based approaches to science education improve critical thinking and engagement of students in coursework along with immersing them in the nature of science (1, 2). Involving undergraduates in authentic research is a variation on inquiry-based classes in line with recent education recommendations (3, 4). Adapting a project from the Csonka laboratory (5), we developed the "Genetic Analysis of Adaptation to Osmotic Stress in *Salmonella*" module in the framework of the Center for Authentic Science Practice in Education (6), which enhances students' scientific understanding and communication skills while introducing them to basic laboratory skills, primary literature, and experimental design. This class has been offered to a total of 32 first-year undergraduate students in 2 years, with 21 additional students currently enrolled this spring.

The objective of the student project was to isolate mutations that alter the regulation or substrate specificity of the ProP protein of *Salmonella enterica* serovar *typhimurium* (see the figure). This protein transports proline and glycine betaine, which regulate the tonicity of the cytoplasm in cells (7, 8). Working in teams of two or three, students isolated spontaneous or mutagen-induced derivatives carrying mutations in ProP that increased the activity of the protein with proline as the transport-substrate. Students carried out classical genetic mapping to confirm that the mutations were in the *proP* gene, amplified the gene and flanking sequences with the polymerase chain reaction, and determined the sequence of the amplicons. Amino acid changes in the mutants were deduced by BLASTN comparisons of the DNA sequence of the amplicons against the

S. typhimurium database sequence. Students obtained mutations resulting in six different amino acid substitutions in the ProP protein (see the figure) and visualized the location of the mutations by constructing three-dimensional (3D) models of ProP (see the figure). At the conclusion of the semester, teams described their results to the class in 10-min PowerPoint presentations and to faculty and students in poster presentations.

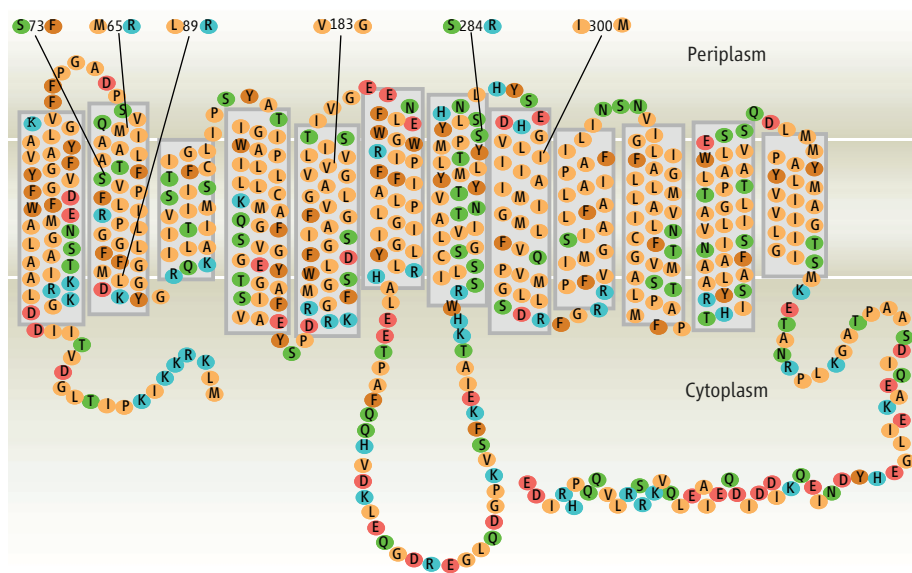
Results obtained in these classes led to a scientific publication in a refereed journal with the students as coauthors (9). The education component of this course model has been presented at several meetings [e.g., (10)].

The intellectual strength of this class is that it introduces the students to the sophisticated mind-set of bacterial genetics, DNA sequencing, and bioinformatic analysis with technically simple and inexpensive experiments. Students see evolution occurring in a Petri dish in a 72-hour time frame. Moreover, they get acquainted with the time-honored practice of classical bacterial genetics of "letting the organism tell you" what is important, without making a priori hypoth-

eses, in this case, which amino acids participate in the regulation or substrate recognition of the ProP protein.

This particular project is sustainable as long as students keep finding new mutations in *proP* or outside of this gene. The specific objectives and experiments can evolve from semester to semester as the project builds on findings from previous students. For example, the current students in the class explored isolating mutants in high-salt environments and using different mutagens, both of which may result in unique mutations. Our selection was designed to identify mutations in *proP*. However, there were mutations in other loci whose identity could be determined by whole-genome sequencing.

We encountered both successes and challenges in involving first-year undergraduates in research projects. With opportunity and guidance, students can be creative in designing experiments and interpreting data, and they immediately see the utility of basic skills, like dilutions and aseptic techniques. Our approach introduces them to several aspects of scholarly activities, including working in teams to solve problems, reading



Amino acid mutations obtained by students (9). The amino acid changes are overlaid on the proposed secondary structure of the *Escherichia coli* K-12 ProP (8), modified to show the 5% sequence divergence of the ProP protein of *S. typhimurium*. Some mutations were isolated multiple, independent times.

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Students constructing a 3D model of the ProP protein.



and discussing primary literature, designing experiments, writing research proposals, communicating results in written and oral formats, and, finally, the process of writing, submission, and review of a refereed, scientific paper. Students left the poster sessions stating that they felt empowered, energized, and accomplished.

Constructing a first-year laboratory course around authentic research involves unique practical considerations. Planning and implementation of the module demand greater instructor time than standard lab

courses. Successful guidance of the students in this specific project requires that the instructors be familiar with standard techniques of bacteriology and phage biology. Another demand comes from overseeing four to six different components of the project. We have found that keeping the class size small (<21 students) and having students work in groups of three allows productive group work while giving each team member an opportunity to practice techniques. Development of the students' ability to communicate their thoughts and experimental findings in written and oral assignments requires extensive feedback and guidance.

Offering this class requires the instructional staff to act as facilitator and mentor to help the students navigate the uncertain nature of research. It is imperative to provide students with clear, specific objectives regarding practical lab skills and the process of science that are introduced on the first day and reinforced throughout the semester. Students must transition from the mentality of getting the "right answer" for a good grade to being thoughtful and careful. Instructors need to communicate that, if experiments were done accurately and carefully—whatever their outcome, data are what they are, and this is considered a success! As such, students are graded on the quality of their thought and method, based on objective evaluation of lab notebooks and written and oral reports, regardless of whether they obtained novel mutations.

We used two methods to assess the impact of our course on student attitudes about science and the development of critical thinking skills. Pre- and postsemester sur-

veys revealed that students recognized that they were involved in the process of discovery with a positive impact on their sense of confidence and interest in conducting scientific research. We used the Critical Thinking Assessment Test developed at Tennessee Tech University to assess the effectiveness of our course in fostering critical thinking skills and observed significant improvement in scores at the end of the semester compared with the beginning. Long-term impact will be evaluated by following the performance and choices of students in this course, compared with students in the traditional lab course, as they move through their undergraduate careers.

First-year research experiences such as these are adaptable at other educational institutions in a variety of subjects by designing simple, low-cost experiments that take advantage of Web resources and faculty expertise. A neurobiology module has been developed using this approach (11). By providing tomorrow's scientists with an early opportunity to contribute to scientific discovery, generate interesting data, and develop their analytical and communication skills, tomorrow begins today.

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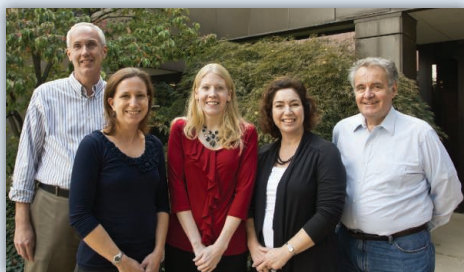
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AAAS ANNUAL MEETING

In a "Flattening" World, Innovation Must Be Global, S&T Leaders Say

VANCOUVER, BRITISH COLUMBIA—With the world's population pushing toward 9 billion by mid-century, humanity must find ways to meet unprecedented needs for food, water, and energy without further damaging the environment. It's a daunting challenge, and experts gathered at the 178th AAAS Annual Meeting said that global research collaborations will be critically important for solving these complex and urgent problems.

In the address that opened the meeting, AAAS President Nina V. Fedoroff described one of these problems in stark relief: Scientists must find ways to double the world's food supply by 2050, the influential plant biologist warned, but major crop yields have declined by roughly 10% for each degree of global climate warming.

"We need to develop crops that thrive in a hotter world on land we now consider unfarmable, using water we now consider unsuitable for agriculture," Fedoroff said. She suggested that the challenge will be met only through innovations born out of stronger partnerships between the developed and developing world.

The problem-solving potential of such collaborations was a recurring theme during the 16 to 20 February meeting in Vancouver, the first to take place outside the United States since 1981. Under the theme "Flattening the World: Building a Global Knowledge Society," more than 11,000 scientists, educators, journalists, and others explored ways to encourage research across borders.

"Much of the responsibility for building and maintaining international research connections falls to research institutions and scientists," wrote University of British Columbia President Stephen Toope and AAAS CEO Alan I. Leshner in a *Vancouver Sun* commentary published before the meeting. "We must expand our international perspective whenever possible, join in multinational projects, and serve as mentors when colleagues in developing nations ask for our help."

The meeting itself was a multinational project in many respects, with participants from some 50 nations and a significant

presence by the event's Canadian hosts. David Lloyd Johnston, the governor-general of Canada, addressed the AAAS crowd at a reception following Fedoroff's address. Canadian astronaut Steve MacLean and Canadian senator and First Nations member



Communication innovation. Hans Rosling used a humorous but compelling prop to explain world population growth at a standing-room-only discussion on public engagement with science. On the right, panelists Frank Sesno (top), Olivia Judson, and James Hansen.

Lillian Eva Dyck gave prominent lectures. AAAS's free Family Science Days drew a record crowd of more than 6400 local children and their parents and teachers.

The meeting offered a range of perspectives on the contributions of science in a flatter world. Sessions explored how new marine reserves can be governed across political boundaries; how surveillance at major ports like Vancouver has kept emerging "superbug" viruses under control; and how breakthroughs in artificial meat production could satisfy the world's growing appetite for beef and pork.

But effective communication also will be essential for building public interest and support for initiatives in climate change and other global issues. The best approaches for public engagement were highlighted in a passionate

and sometimes raucous discussion, moderated by former CNN anchor Frank Sesno and featuring NASA Goddard Institute for Space Studies head James Hansen, international health statistician Hans Rosling, and author and biologist Olivia Judson.

Scientists must experiment with new techniques to deliver the message of climate change, the participants agreed, but they also suggested that a more fundamental shift is necessary. "What I would like to try and change is not so much the way people understand the facts," Judson said, "but the way they

look at the world and ask questions about it."

The meeting's participants also addressed social and political implications of global scientific challenges, including the impact of the Arab Spring movement and reverberations from Japan's Fukushima nuclear disaster.

Ismail Serageldin, director of Egypt's Bibliotheca Alexandrina, said science's principles of "tolerance and rationality" can guide his nation and others in the region as they rebuild their governments. The best "defense against extremists is not by

censorship or autocracy," he said in a videotaped plenary address. "It is by embracing pluralism, and defeating ideas with ideas—and here science has much to say."

The competition of ideas also fuels scientific innovation, said Canadian entrepreneur and BlackBerry founder Mike Lazareidis. But in his plenary speech, he suggested that governments and businesses must invest in ideas without obvious applications, even when practical solutions are needed for a host of global challenges.

History tells us, Lazareidis said, that it's impossible to guess at the impact of today's scientific breakthroughs and to predict which will solve the planet's emerging threats. But "it's the ideas themselves that got us this far," he concluded, "and it's new ideas that will get us even further."

—Becky Ham

Science & Diplomacy—A New AAAS Quarterly

With interest in science diplomacy growing worldwide, AAAS has debuted an online, quarterly publication intended to build dialogue between the science and foreign policy communities and to encourage the intellectual development of such diplomacy in a variety of forms.

Science & Diplomacy [www.sciencediplomacy.org] was launched by the AAAS Center for Science Diplomacy just days after the 40th anniversary of the Shanghai Communiqué, in which Chinese Premier Zhou Enlai and U.S. President Richard Nixon signaled that the two Cold War rivals should normalize relations and that science and technology were areas critical to building understanding.

Today, many of the most important issues facing humanity are regional and global in nature, and a new generation of science diplomacy is building relations between nations and supporting international research cooperation.

The first issue of *Science & Diplomacy*



Transforming history. Forty years ago, U.S. President Richard Nixon (left) and Chinese Premier Zhou Enlai signed the Shanghai Communiqué, which identified science as one way to build mutual understanding.

includes articles by senior scientists, diplomats, and policy-makers, including U.S. Senator Richard Lugar (R–Indiana), ranking member of the Senate Foreign Relations Committee and an expert in arms control; Robert D. Hormats, U.S. under secretary of state for economic growth, energy, and the environment; South African Science and

Technology Minister Naledi Pandor; and Alice P. Gast, Lehigh University president and U.S. science envoy to Azerbaijan, Kazakhstan, and Uzbekistan.

Editor-in-Chief Vaughan C. Turekian, who also directs the Center for Science Diplomacy, and senior advisory board chairman Norman P. Neureiter, who was the first science adviser to the U.S. secretary of state, said that the new publication will be a resource for foreign policy professionals, scientists and research administrators, journalists, educators, and students.

“We believe the time is right to catalyze greater thought and discussion about issues at the interface of science and diplomacy,” they write in the first issue. “Our goal is a foreign policy that can fully address the increasingly complex technical dimensions of 21st century international relations.”

The AAAS Center for Science Diplomacy, founded in 2008, has emerged as a leading international influence in the field. The publication was developed with the financial support of the Golden Family Foundation.

—Caitlin Jennings and Edward W. Lempinen

WORKFORCE DEVELOPMENT

A “Call to Action” on Minority Men in Science

The numbers tell a troubling story: Between 9th and 10th grades, about 25% of African American and Hispanic young men drop out of school. Among those who enroll in college and study science-related fields, nearly one-fifth work 20 or more hours a week to make ends meet. Perhaps it is no surprise, then, that while African American, Hispanic, and Native American men in 2008 accounted for 35% of the college-age male population in the United States, they received only about 12% of bachelor’s degrees in science-related fields.

Some education experts have struggled for years to understand and address the low numbers of minority men in science, technology, engineering, mathematics—the STEM fields. Today, there is growing agreement that, if the United States is to build a bigger, stronger science-skilled workforce, it must cultivate talent across the full population. The question is: What are the most effective strategies?

At a 28 February symposium sponsored by the Association of Public and Land-Grant Universi-

ties (APLU), the National Aeronautics and Space Administration (NASA), and AAAS, participants explored recent research that offers some clear, practical guidance.

“We identified the challenges and best practices,” Leland Melvin, NASA’s associate administrator for education, told the audience convened at his agency’s Washington, D.C., headquarters. “Now it is time to go the next step and really have a call to action to increase minority participation in STEM fields.”

A key focus of the day-long event was a new APLU report, “The Quest for Excellence: Supporting the Academic Success of Minority Males in Science, Technology, Engineering, and Mathematics (STEM) Disciplines.” Based on a survey of more than 1600 minority students, higher education faculty, and administrators, it drew a sharp set of conclusions:

To succeed in science-related studies and professions, motivated men from underrepresented minority groups need active engagement and mentoring by college faculty, hands-on involve-

ment in undergraduate research, and adequate financial support. Many successful students credit rigorous Advanced Placement classes in high school with preparing them for college. And many faculty and administrators say that their schools need a deeper commitment to the success of minority men.

Shirley Malcom, director of Education and Human Resources at AAAS, told the symposium audience that schools need to collect better data on minority students to see not only who is admitted, but what they study, and what they do after leaving school.

Students who overcome the challenges find a range of rewards, said Nicole Smith, a senior economist at Georgetown University’s Center on Education and the Workforce. Earnings are relatively high. And compared with other sectors of the workforce, there is less of a pay gap between minorities and women on one side and white men on the other. In the United States, Smith said, science “is one of only a few equal-opportunity occupations.” —Warren Leary

Results of the 2011 Election of AAAS Officers

Following are the results of the 2011 election. Terms began on 20 February 2012.

General Election

President: Phillip A. Sharp
Board of Directors: Bonnie L. Bassler; May R. Berenbaum

Committee on Nominations: Mary-Claire King; Barbara J. Meyer; Cherry A. Murray; Keith R. Yamamoto

Section on Agriculture, Food, and Renewable Resources

Chair Elect: Susan R. McCouch
Member-at-Large of the Section Committee: Jo Handelsman
Electorate Nominating Committee: Candace H. Haigler; Gregory B. Martin
Council Delegate: Maud A. W. Hinchey

Section on Anthropology

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Electorate Nominating Committee: David G. Anderson; Leslea J. Hlusko

Section on Astronomy

Chair Elect: Sandra M. Faber
Member-at-Large of the Section Committee: Megan Donahue
Electorate Nominating Committee: Martha P. Haynes; Bruce Margon

Section on Atmospheric and Hydrospheric Sciences

Chair Elect: Andrew Dessler
Member-at-Large of the Section Committee: Alan Mix
Electorate Nominating Committee: Margaret Leinen; Joyce E. Penner

Section on Biological Sciences

Chair Elect: Joy Bergelson
Member-at-Large of the Section Committee: Ellen D. Ketterson
Electorate Nominating Committee: Sarah Hake; Jeannie T. Lee

Section on Chemistry

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Member-at-Large of the Section

Committee: Melanie Sanford

Electorate Nominating Committee: Anna K. Mapp; William B. Tolman

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Chair Elect: Paul H. Krebsbach
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Electorate Nominating Committee: Anne George; J. Timothy Wright

Section on Education

Chair Elect: Samuel M. Taylor
Member-at-Large of the Section Committee: Dale Rose Baker
Electorate Nominating Committee: Julia V. Clark; Judy Scotchmoor

Section on Engineering

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Section on General Interest in Science and Engineering

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Section on Geology and Geography

Chair Elect: Tim Dixon
Member-at-Large of the Section Committee: Naomi Oreskes
Electorate Nominating Committee: Brenda Ekwurzel; Walter D. Mooney

Section on History and Philosophy of Science

Chair Elect: Davis Baird
Member-at-Large of the Section Committee: Naomi Oreskes
Electorate Nominating Committee:

Thomas Nickles; Robert T. Pennock
Council Delegate: Vassiliki Betty Smocovitis

Section on Industrial Science and Technology

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Electorate Nominating Committee: Toni Carbo; Tom Dietterich

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Electorate Nominating Committee: Mark Aronoff; Eric Potsdam

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Section on Pharmaceutical Sciences

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Electorate Nominating Committee: Alice M. Clark; James M. Gallo

Section on Physics

Chair Elect: Jay Marx
Member-at-Large of the Section Committee: David K. Campbell
Electorate Nominating Committee: Angela V. Olinto; Stuart Parkin

Section on Psychology

Chair Elect: Richard N. Aslin
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Council Delegate: Lewis P. Lipsitt

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Electorate Nominating Committee: Lynda T. Carlson; Jonathan D. Mayer
Council Delegate: Amy O. Tsui

Section on Societal Impacts of Science and Engineering

Chair Elect: Susan E. Cozzens
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Electorate Nominating Committee: David H. DeVorkin; Doris Teichler Zallen

Section on Statistics

Chair Elect: Sally C. Morton
Member-at-Large of the Section Committee: Jessica Utts
Electorate Nominating Committee: Hans-Georg Müller; Javier Rojo

Integrated Electromicrobial Conversion of CO₂ to Higher Alcohols

Han Li,^{1,2} Paul H. Opgenorth,³ David G. Wernick,¹ Steve Rogers,⁴ Tung-Yun Wu,¹ Wendy Higashide,⁴ Peter Malati,⁵ Yi-Xin Huo,⁴ Kwang Myung Cho,⁴ James C. Liao^{1,2,3,6*}

A man-made photovoltaic device is relatively efficient in converting sunlight to electricity, but the electrical energy generated is difficult to store. The biological photo-systems, on the other hand, are limited by the intrinsic design and biomaterials available, for which no near-term improvements are in sight (1). One way to circumvent both problems is to link man-made solar cells to biological CO₂ fixation and fuel production. Theoretically, H₂ generated by solar electricity can drive CO₂ fixation in lithoautotrophic microorganisms engineered to synthesize high energy-density liquid fuels. However, the low solubility, low mass transfer rate, and safety issues of H₂ in microbial cultures limit the efficiency and scalability of such processes.

Compared with H₂, formic acid is a favorable energy carrier. Electrochemical production of formic acid from CO₂ and H₂O can achieve relatively high efficiency (2, 3). Formate is highly soluble and is readily converted to CO₂ and NADH in the cells, providing a safe replacement for H₂. However, the high solubility of formate increases the cost of separation. Accumulated formate will decompose at the anode, decreasing the yield of the process (2). Therefore, simultaneous electrochemical formate production and biological formate conversion to higher alcohols is desirable (Fig. 1A). Unfortunately, introduction of electricity to microbial cultures may impede cell growth.

As such, an integrated process for reduction of CO₂ to liquid fuel powered by electricity requires (i) metabolic engineering of a lithoautotrophic organism to produce liquid fuels, (ii) electrochemical generation of formate from CO₂ in fermentation medium, and (iii) enabling mi-

crobes to withstand electricity. In this work, we chose *Ralstonia eutropha* H16 as the production host and isobutanol and 3-methyl-1-butanol (3MB) as the target fuels, which can be used in the internal combustion engines. We introduced the set of genes previously reported (4, 5) for isobutanol and 3MB production into *R. eutropha* H16 (fig. S1, A to E). We also disrupted polyhydroxybutyrate synthesis (fig. S1, A and B) and used the heterologous isobutanol and 3MB production pathway as the new metabolic sink for carbon and reducing equivalents. In a pH-coupled formic acid feeding fermentor, the engineered strain LH74D produced fuels with the final titer of over 1.4 g/l (~846 mg/l isobutanol and ~570 mg/l 3MB) (fig. S2B).

Next, we used an In foil cathode and a Pt anode in the culture medium bubbled with air containing 15% CO₂ to produce formate electrochemically (Fig. 1A). However, growth of *Ralstonia* was inhibited when electric current was introduced. Studies using *Escherichia coli* showed transient inhibition of electric current on cell growth (fig. S3). When the current stopped, cell growth resumed, suggesting that unstable compounds such as reactive oxygen or nitrogen species might be responsible for the growth inhibition.

To test this hypothesis, we constructed three *Ralstonia* strains with different reporter plasmids to detect the presence of reactive oxygen and nitrogen species. The plasmids each contained a *lacZ* gene driven by a promoter of *Ralstonia* *katG*, *sodC*, or *norA* gene, genes which have been shown to be induced by hydrogen peroxide (H₂O₂), superoxide free radicals (O₂⁻), and nitric oxide (NO), respectively (6–8). When the plasmid-bearing

strains were exposed to electrolysis, expression of β-galactosidase from *sodC* and *norA* promoters were induced but not from the *katG* promoter (Fig. 1B). These results suggested that O₂⁻ and NO trigger a stress response in *Ralstonia* cells and inhibit growth. To circumvent this toxicity problem, a porous ceramic cup was used to shield the anode (Fig. 1A). This inexpensive shield provides a tortuous diffusion path for chemicals. Therefore, the reactive compounds produced by the anode may be quenched before reaching the cells growing outside the cup. Using this approach, healthy growth of *Ralstonia* strain LH74D and production of over 140 mg/l biofuels were achieved with the electricity and CO₂ as the sole source of energy and carbon, respectively (Fig. 1C).

This integrated process to convert CO₂ to liquid fuels does not depend on biological “light reactions.” Electricity generated from photovoltaic cells, wind turbines, or off-peak grid power sources can be used to drive CO₂ fixation and fuel production. Thus, this process provides a way to increase photosynthetic efficiency by coupling man-made photoelectric generation device with biological CO₂ fixation and fuel production capability. The approach demonstrated here can also be applied to produce other compounds, thus opening the possibility of electricity-driven bio-conversion of CO₂ to a variety of chemicals. Furthermore, because formate is a by-product of chemical dehydration and conversion of biomass (9), transformation of formate into liquid fuel will also play an important role in the biomass refinery process.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S4
Tables S1 and S2
References (10–22)

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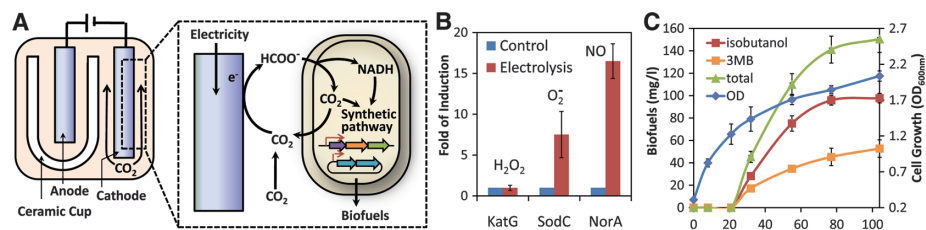


Fig. 1. An integrated electromicrobial process to convert CO₂ to higher alcohols. (A) Electricity powered the electrochemical CO₂ reduction on the cathode to produce formate, which is converted to isobutanol and 3MB by the engineered *R. eutropha*. (B) *R. eutropha* strains harboring a reporter gene driven by promoters that respond to reactive oxygen and nitrogen species showed increased reporter activity upon electrolytic exposure. (C) Engineered strain *R. eutropha* LH74D (supporting online material) showed healthy growth and produced over 140 mg/l biofuels in the integrated electromicrobial reactor with electricity and CO₂ as the sole energy and carbon sources, respectively. Error bars indicate SD (*n* = 3).

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Emerging Chirality in Artificial Spin Ice

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Artificial spin ice, made up of planar nanostructured arrays of simple ferromagnetic bars, is a playground for rich physics associated with the spin alignment of the bars and spin texture associated with the magnetic frustration at the bar vertices. The phase diagram is exotic, showing magnetic monopole-like defects and liquid and solid phases of spins arranged in loop states with predicted chiral order. We show that magnetotransport measurements in connected honeycomb structures yield the onset of an anomalous Hall signal at 50 kelvin. The temperature scale can be attributed to the long-range dipolar ice phase. The topological Hall signal arises because chiral loops form at the sample edges, indicating a generic route to exotic states via nanoarray edge structure.

Frustrated magnets have complex spin structures. Spin ices are a class of frustrated magnets in which the spin disorder resembles the proton disorder in water ice (1–3). In addition to naturally occurring compounds, artificial spin-ice nanostructures have been built with single-domain ferromagnetic islands, or bars, that act as macrospins with dipolar interactions. These form local order by short-range frustration (4–6). We study honeycomb structures, sometimes called kagome spin ice (7), in which the consideration of longer-range correlations has led to the prediction of exotic phases (6, 8) that have specific spin texture (i.e., characteristic features in the specific bar spin arrangements).

A uniformly magnetized bar can be considered as a dumbbell of magnetic charge (9). In artificial spin ice, if the dumbbell length l is smaller than the lattice spacing a (Fig. 1A), then the uneven charge distribution around the vertex (Fig. 1B) gives rise to a vertex dipole moment (VDM), as shown in Fig. 1C. In honeycomb structures, all possible low-energy vertex configurations (a to f in Fig. 1D) have finite magnetic charge and VDM. Theoretical analysis of the system yields (6, 8) a rich phase diagram (Fig. 1E). The macrospin correlations are zero in the gas-like Ising paramagnet, nearest neighbor only in the liquid-like short-range spin ice (Ice I), near neighbor in the distinct liquid-like (long-range) spin ice phase (Ice II), and infinite in the fully ordered ground state. The Ice I to Ice II phase transition is chiral (6); at the transition a number of mobile closed loops of each chirality form. Further spin flips in the Ice II system conserve the populations of clockwise and anticlockwise loops. However, the phases have proved inaccessible experimentally because the system remains trapped in a metastable state and the ground state is hidden (10–13).

Spin on the conduction electrons follows the local magnetization direction, and chirality in the

spin texture imparts to them a phase called the Berry phase. This mechanism produces an anomalous Hall signal called the topological Hall effect (14–17). It is an exquisitely sensitive probe of the spin chiral order parameter (18) requiring a net imbalance in either total chirality or its spatial

distribution. Connected honeycombs naturally lend themselves to interrogation by such methods. Here we search for Hall and magnetoresistance (MR) (19) signatures of topological effects by exploring the transport response to swept magnetic fields. The results are most clearly observed with parallel Hall geometry, where the switching transition states of the array in the forward quadrant of the magnetization loop are measurably different from any state realized in the backward quadrant.

Our electrically continuous cobalt honeycomb covers an area 100 μm by 100 μm square and is composed of bars 1 μm long, 100 nm wide, and 20 nm high. The four-point technique for measuring magnetotransport in large-area arrays (20, 21) is described in the supplementary methods. The raw magnetotransport data are shown in Fig. 2 for five different relative orientations of current, voltage, field, and honeycomb as indicated in the schematics. The data were taken at 100 K (Fig. 2, A to E), 2 K (Fig. 2, F to I), and 25 K for the standard Hall (Fig. 2J). The MR

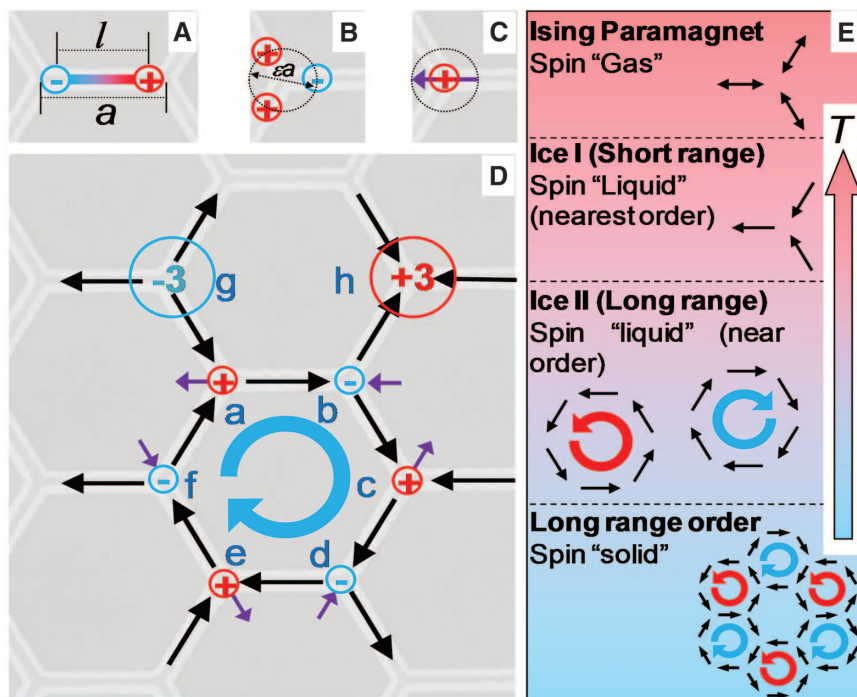


Fig. 1. Magnetic vertex configurations and equilibrium phase diagram for artificial spin ice honeycombs. The background (A to D) is a scanning electron micrograph of a small section of the array. (A) The magnetic charge $\pm q = \pm m/l$ dumbbell representation of a magnetic moment m of length l on a honeycomb of lattice parameter a . Deviations from ideal Ising behavior are characterized by $\epsilon = 1 - l/a$. (B) Charges on neighboring dumbbells reside on the circumference of a circle of diameter ϵa . This is equivalent to (C) a point charge $Q = \Sigma q$ and a vertex dipole moment (VDM) of magnitude $\epsilon a \Delta q/2$ colocalized at the vertex center. ($\Delta q = q_{\text{max}} - q_{\text{min}}$ at the vertex.) In connected arrays, the local magnetic structure at the vertex minimizes the VDM (21). (D) The eight possible configurations of magnetic moments (black arrows) at a kagome spin ice vertex. Vertices a to f obey the ice rule and have $Q = \pm q$; VDM = $\epsilon a q$ (purple arrows) and spin chirality $\Omega = -1/3$; vertices g and h are ice rule defects with $Q = \pm 3q$; VDM = 0 and $\Omega = +1$ (21). (E) The predicted phase diagram for artificial spin ice honeycombs (6, 8) (only the Ice I phase is observed in zero-field). There are four distinct phases: the gas-like Ising paramagnet; the liquid-like short-range spin ice (Ice I) phase; the long-range spin ice (Ice II) phase where the near order extends to second nearest neighbor macrospins, giving favored vertex pairs [ab], [cd], and [ef]; and the long-range ordered macrospin solid state.

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and Hall components of the magnetotransport were separated (22) by virtue of their symmetry with respect to the applied field. The reversible and irreversible components (respectively the sum and the difference between the forward and backward field sweeps) of the parallel Hall resistance are shown in Fig. 3, A and B.

All the transport phenomena observed at 100 K (and above) are straightforward consequences of the lattice geometry and the standard transport effects of ferromagnetic metals. The 100 K MR data in Fig. 2, A to C, are similar to those for a previous permalloy honeycomb (19) [with two inequivalent current directions, “armchairs” (Fig. 2,

A and F) and “zigzags” (Fig. 2, B and G)]. The vertices are complex magnetic structures containing a vertex domain wall (VDW), the magnetization of which can be either parallel or perpendicular to the current. The irreversible magnetoresistance at 100 K is simply the anisotropic magnetoresistance (AMR) of ferromagnetic metals, and the magnitude is determined by the number of vertices whose VDW magnetization is perpendicular to the current direction (the low-resistance state). The maximum magnitude of the effect ($\Delta R = 0.1$ ohm) complements recent measurements on single perpendicular transverse domain walls in a single nanobar ($\Delta R = 0.23$ ohm) (23), which is expected as the maximum possible AMR in the array would be one perpendicular VDW per nanobar, and the array is square.

Let us now consider the low-temperature effects (Fig. 2, F to J). New features emerge in all configurations but are seen most clearly in parallel Hall geometry (Fig. 2I) because the current and field are nominally parallel, eliminating conventional Hall effects. At 100 K (Fig. 2D) there is zero signal with Hall symmetry, only mixed-in AMR. However, at 2 K a peak appears in the field range associated with magnetic switching (Fig. 4A). The peak feature is hysteretic (irreversible) and has Hall symmetry. We have observed the onset of a parallel Hall signal in a honeycomb array fabricated from a thin cobalt film from a different source (21), so these are not the results of a specific sample; no parallel Hall component is observed in unpatterned films (24). The symmetrized reversible and irreversible parallel Hall components are plotted versus field at selected temperatures in Fig. 3, A and B, respectively. The two components have opposite sign, and different magnitude, but their thermal evolution is markedly similar: The maximum value of each tends to zero at around 50 K (Fig. 3B, inset). As there is no out-of-plane field, no out-of-plane magnetization, and no angle between the field and average current direction, the clear handedness in the transverse deflection of the carriers is direct evidence of spin-chiral asymmetry emerging below about 50 K. The hysteretic peak effect mid-transition bears a strong similarity to that observed in the itinerant spin-liquid pyrochlore $\text{Pr}_2\text{Ir}_2\text{O}_7$ when the field is applied along the [111] (kagome ice) direction (18).

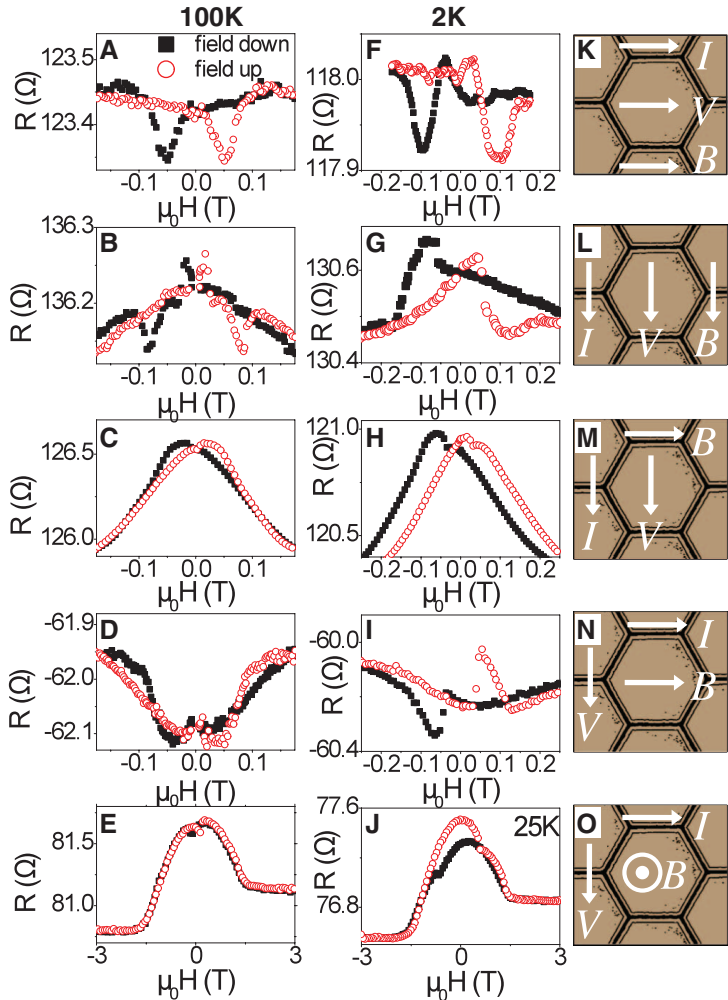
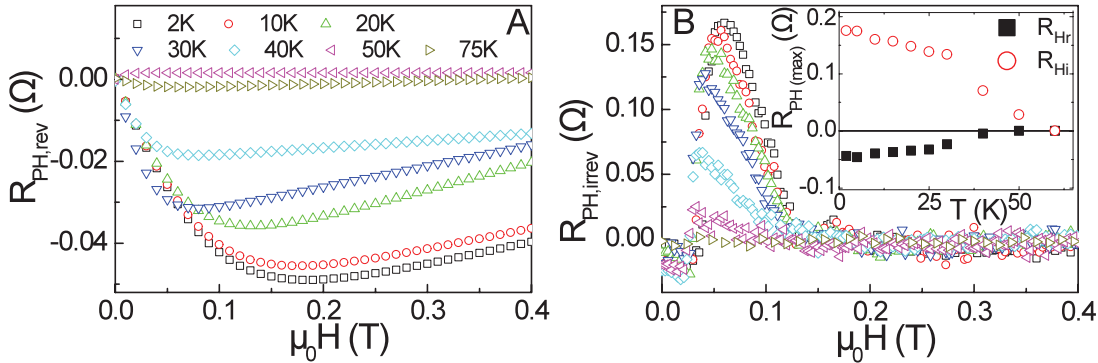


Fig. 2. Raw magnetotransport data. Resistance versus field with positive (filled squares) and negative (open circles) sweep direction. Data were taken at 100 (A to E), 2 (F to I), and 25 K (J). Schematics of measurement geometries: (K) longitudinal (armchair) MR geometry in (A) and (F); (L) longitudinal (zigzag) MR geometry in (B) and (G); (M) transverse MR geometry in (C) and (H); (N) parallel Hall geometry in (D) and (I); and (O) standard Hall geometry in (E) and (J).

Fig. 3. Topological Hall signal and possible sources of spin chirality in the kagome spin ice structure. (A) Reversible Hall symmetry component and (B) irreversible Hall symmetry component of the parallel Hall resistance (R_{PH}) versus applied field at selected temperatures from 2 to 75 K. Inset (B): Temperature dependence of the saturation reversible and peak irreversible parallel Hall resistance.



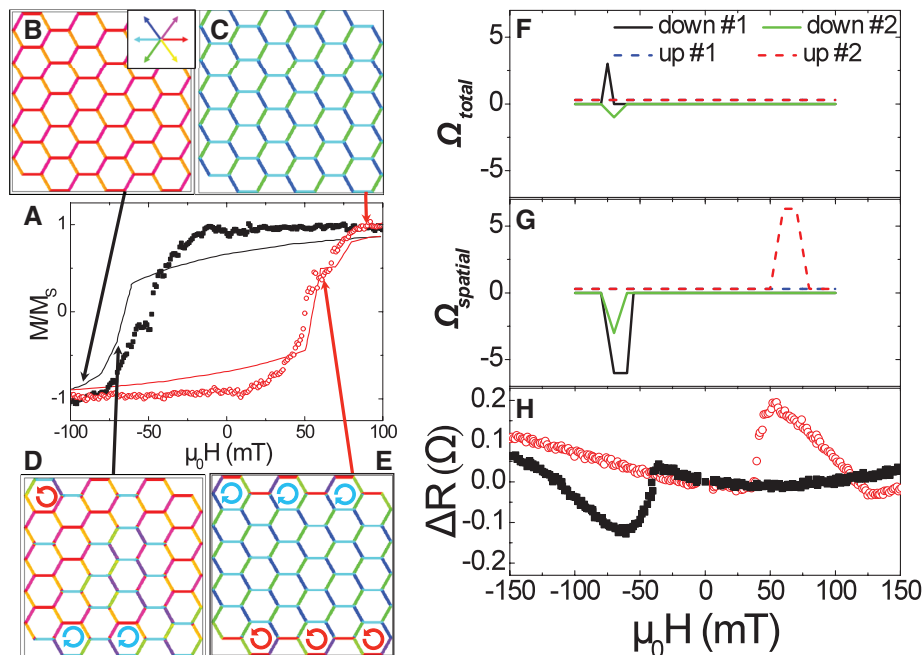


Fig. 4. Spatial separation of chirality: **(A)** Normalized magnetization of honeycomb at 100 K versus field swept in the negative direction (black solid squares) and positive direction (red open circles), as determined from longitudinal AMR measurements. Solid lines show the negative (black) and positive (red) MH curves calculated by OOMMF (25) for 6×5 arrays. **(B to E)** OOMMF simulations at a selected point in the hysteresis loop indicated by the straight arrows to Fig. 4A. The magnetization direction of each bar is given by the color-wheel [inset in (B)]; clockwise and anticlockwise loops in the magnetization are indicated by blue and red circular arrows, respectively. **(F)** Total loop chirality ($\Omega_{total} = A - C$) and **(G)** spatial loop chirality [$\Omega_{spatial} = (A_{bottom} - A_{top}) - (C_{bottom} - C_{top})$] where C and A are the number of clockwise and anticlockwise loops, respectively, at each field in two nominally identical OOMMF simulations. Up sweeps (dashed) have been offset slightly from the down sweeps (solid lines) for clarity. **(H)** Change in 2 K parallel Hall resistance [$\Delta R = R_H - R_H(0 \text{ T})$] versus field in the negative sweep direction (black squares) and positive sweep direction (red circles). The topological Hall effect of the loops observed in (D) (C_{bottom} and A_{top}) pushes carriers near the edges back toward the center (decreasing R_H), and loops shown in (E) (C_{top} and A_{bottom}) drive carriers into the edge (increasing R_H).

To investigate the source of the topological Hall signal, we performed zero-temperature micromagnetic simulations using the Object Oriented Micromagnetic Framework (OOMMF) code (21, 25). The simulated arrays were 6×5 hexagons with the same bar dimensions and corrugated edges as the sample (fig. S2). The field was swept from magnetic saturation in the positive x direction to negative saturation, and then back to positive saturation in 10-mT steps. The simulated MH (magnetization versus field) loop is compared to experiment in Fig. 4A and captures the switching fields well. The micromagnetic configurations showed a tendency to form loop states with substantial vector spin chirality at the edges during switching (Fig. 4, B to E). The net asymmetry across the whole array is investigated by counting loops at each field point and then defining both a total loop chirality and a spatial loop chirality, shown in Fig. 4, F and G, respectively. Ferromagnetic switching is a stochastic process, so we repeated the full loop with identical input parameters. The randomness in both quantities is much more pronounced in the small simulated arrays than in the experiment. However, whereas the total chirality (Fig. 4F)

fluctuates randomly around zero, the spatial chirality gives the field asymmetric peak effect (Fig. 4G) observed in the 2 K parallel Hall resistance data (ΔR , Fig. 4H). Magnetic switching occurs by nucleation and propagation of domain walls. Nucleation is favored at the left and right sample edges. The domain walls tend to propagate along the field direction, rather than moving to the top and bottom edges of the sample, so these edge bars are often the last to switch. As a result, clockwise and anticlockwise loops tend to form at the top and bottom edges, and the handedness is inverted in the opposite sweep direction. The sign of the topological Hall effect is such that the loops observed in the backward sweep direction (Fig. 4D) (C_{bottom} and A_{top}) push carriers near the edges back toward the center and those in the forward sweep (Fig. 4E) (C_{top} and A_{bottom}) drive the conduction electrons further toward the edge. The simulation, without finite temperature or structural imperfections, simulates magnetic switching sharper than the data.

Micromagnetic simulations offer an explanation of the chiral asymmetry at base temperature, but not of the thermal evolution of the topological

Hall components below 50 K (Fig. 3B, inset). The cobalt Curie temperature, 1400 K, is far removed, suggesting the scale is ice related, with the chiral (6) phase transition from Ice I to Ice II a promising candidate. Using the simulated magnetic charge distribution, we estimate the VDM of our structure, and so obtain an Ice II transition temperature (6) of ~ 50 K (21). Constrained dynamics in artificial spin ice (10, 11, 26) prevent the phase transition, without suppressing its expression in the topological Hall signal, via the irreversible selection of chiral intermediates in the switching process. Recent predictions indicate that constrained dynamics may in fact help support the topological Hall asymmetry (26). The VDM is dependent on bar material (4, 27) dimension and separation in disconnected kagome structures (13, 28), enabling the temperature scale associated with these topological states to be tuned. We expect our result to be relevant for controlled investigation of other emerging magnetic degrees of freedom such as monopoles (4, 9, 27), loops (6), and topological edge (29, 30) states. We have shown that field-driven dynamics allow thermal scales to be accessed experimentally in a system far from thermal equilibrium, an important step toward application of magnetic nanostructures as model frustrated systems and potential devices based on states, such as neural networks (31).

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Materials and Methods

Figs. S1 to S4

References (32–35)

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Disentangling the Electronic and Phononic Glue in a High- T_c Superconductor

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Unveiling the nature of the bosonic excitations that mediate the formation of Cooper pairs is a key issue for understanding unconventional superconductivity. A fundamental step toward this goal would be to identify the relative weight of the electronic and phononic contributions to the overall frequency (Ω)-dependent bosonic function, $\Pi(\Omega)$. We performed optical spectroscopy on $\text{Bi}_2\text{Sr}_2\text{Ca}_{0.92}\text{Y}_{0.08}\text{Cu}_2\text{O}_{8+\delta}$ crystals with simultaneous time and frequency resolution; this technique allowed us to disentangle the electronic and phononic contributions by their different temporal evolution. The spectral distribution of the electronic excitations and the strength of their interaction with fermionic quasiparticles fully account for the high critical temperature of the superconducting phase transition.

Lattice vibrations (1) and excitations of electronic origin, such as spin or electric polarization fluctuations (2) and loop currents (3), are generally considered potential mediators of Cooper pairing in the copper oxide high-temperature superconductors (cuprates). The generic interaction of fermionic quasi-particles (QPs) with bosonic excitations is accounted for by the bosonic function $\Pi(\Omega)$ [usually indicated as $\alpha^2F(\Omega)$ for phonons and $I^2\chi(\Omega)$ for spin fluctuations], a dimensionless function that depends on the density of states of the excitations and the strength of their coupling to QPs. Because both the energy dispersion and lifetime of QPs are

strongly affected by the interactions, signatures of QP-boson coupling have been observed in experiments that probe the electronic properties at equilibrium. The ubiquitous kinks in the QP dispersion at ~ 70 meV, measured by angle-resolved photoemission spectroscopy (ARPES) (4), have been interpreted in terms of coupling to either optical Cu-O lattice modes (5, 6) or spin excitations (7). Inelastic neutron and x-ray scattering experiments found evidence for both QP-phonon anomalies (8) and bosonic excitations attributed to spin fluctuations (7, 9) and loop currents (10). Dip features in tunneling experiments have been used to alternatively support the scenarios of dominant electron-phonon interactions (11) or antiferromagnetic spin fluctuations (12). The frequency-dependent dissipation of the Drude optical conductivity, $\sigma(\omega)$, measured by equilibrium optical spectroscopies, has been interpreted (13–15) as the coupling of electrons to bosonic

excitations, in which the separation of the phononic and electronic contributions is impeded by their partial coexistence on the same energy scale (< 90 meV).

We have disentangled the electronic and phononic contributions to $\Pi(\Omega)$ through a nonequilibrium optical spectroscopy, in which femtosecond time resolution is combined with an energy resolution smaller than 10 meV over a wide photon energy range (0.5 to 2 eV). Our approach can be rationalized on the basis of the widely used assumption (16, 17) that, after the interaction between a superconductor and a short laser pulse (1.55 eV photon energy), the effective electronic temperature (T_e) relaxes toward its equilibrium value through energy exchange with the different degrees of freedom that linearly contribute to $\Pi(\Omega)$. In a more formal description, the total bosonic function is given by

$$\Pi(\Omega) = \Pi_{\text{be}}(\Omega) + \Pi_{\text{SCP}}(\Omega) + \Pi_{\text{lat}}(\Omega) \quad (1)$$

where Π_{be} refers to the bosonic excitations of electronic origin at the effective temperature T_{be} , Π_{SCP} to the small fraction of strongly coupled phonons (SCPs) at T_{SCP} (16), and Π_{lat} to all other lattice vibrations at T_{lat} . Because the term that couples the rate equations (18) for T_e and T_b (with $b = \text{be, SCP, lat}$) is $G(\Pi_b, T_b, T_c)/C_b$ [where G is the functional described in (18) and C_b is the specific heat of the bosonic population], each subset of the bosonic excitations is characterized by different relaxation dynamics on the femtosecond time scale. The most convenient systems for such an experiment are the hole-doped cuprates close to the optimal dopant concentration needed to attain the maximum critical temperature T_c , in which the total $\Pi(\Omega)$ is maximum (15). Despite the magnitude of $\Pi(\Omega)$, vertex corrections beyond

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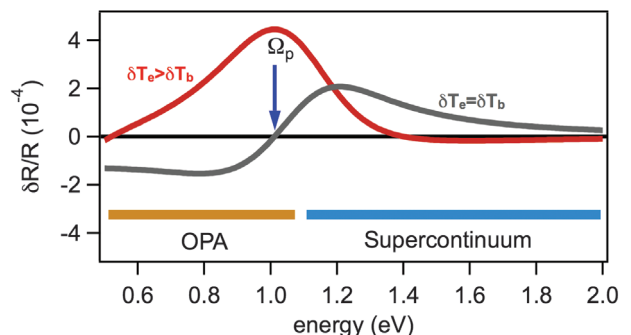
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Fig. 1. Nonequilibrium reflectivity. The relative reflectivity variation $\delta R/R(\omega, T) = [R(\omega, T + \delta T) - R(\omega, T)]/R$, calculated for the incremental variations δT_e and δT_b in Eqs. 2 and 3 and using the $\Pi(\omega)$ value obtained from the fit to the equilibrium measurements, is reported. The change of sign of $\delta R(\omega, T)/R$ in the quasi-thermal scenario (gray line; $\delta T_e = \delta T_b = 1$ K) and the maximum $\delta R(\omega, T)/R$ in the non-thermal scenario (red line; $\delta T_e = 5$ K, $\delta T_b = 0$) occur at a frequency coinciding with the dressed plasma frequency, $\Omega_p \sim 1$ eV. The spectral regions probed by the optical parametric amplifier (OPA) and supercontinuum techniques (18) are indicated.



Eliashberg theory can be reliably neglected (9, 15) in this doping regime.

The total bosonic function $\Pi(\Omega)$ is directly determined by fitting an extended Drude model (18) and a sum of Lorentz oscillators accounting for the interband optical transitions in the visible region (19) to the equilibrium dielectric function of optimally doped $\text{Bi}_2\text{Sr}_2\text{Ca}_{0.92}\text{Y}_{0.08}\text{Cu}_2\text{O}_{8+\delta}$ (Y-Bi2212) high-quality crystals (20) ($T_c = 96$ K) measured at $T = 300$ K by conventional spectroscopic ellipsometry (21). If we assume a histogram-like form (18) (fig. S1) and impose an upper limit of 1 eV, the extracted $\Pi(\Omega)$ is characterized by (i) a low-energy part (up to 40 meV) compatible with the coupling to acoustic phonons (22) and Raman-active optical phonons involving c -axis motion of the Cu ions (23); (ii) a narrow, intense peak centered at ~ 60 meV, attributed to the anisotropic coupling to either out-of-plane buckling and in-plane breathing Cu-O optical modes (6) or bosonic excitations of electronic origin such as spin fluctuations (7); and (iii) a broad continuum extending up to 350 meV (13–15), well above the characteristic phonon cutoff frequency (~ 90 meV).

The key point to extend this analysis to nonequilibrium experiments is that the electron self-energy, $\Sigma(\omega, t)$, used to calculate $\sigma(\omega, t)$ (18), can be factorized into

$$\Sigma(\omega, T) = \int_0^\infty \Pi(\Omega) L(\omega, \Omega, T) d\Omega \quad (2)$$

(24), where $L(\omega, \Omega, T)$ is a material-independent kernel function accounting for the thermal activation of the bosonic excitations and of the QPs. The kernel function is given by

$$L(\omega, \Omega, T_{e,b}) = -2\pi i \left[N(\Omega, T_b) + \frac{1}{2} \right] + \Psi \left[\frac{1}{2} + \frac{i(\Omega - \omega')}{2\pi T_e} \right] - \Psi \left[\frac{1}{2} - \frac{i(\Omega + \omega')}{2\pi T_e} \right] \quad (3)$$

where Ψ is the digamma function obtained by integrating the Fermi-Dirac functions, and $N(\Omega, T_b)$ is the Bose distribution at temperature T_b . The kernel function can be decomposed into different

terms depending on the electronic (T_e) and bosonic (T_b) temperatures. The independent variation of $T_{e,b}$ is expected to induce different modifications of the dielectric function. Figure 1 shows the expected relative variation of the reflectivity, expressed as

$$\frac{\delta R}{R}(\omega, T) = \frac{R(\omega, T + \delta T) - R(\omega, T)}{R(\omega, T)} \quad (4)$$

in the quasi-thermal ($\delta T_e = \delta T_b > 0$) and non-thermal ($\delta T_e > 0, \delta T_b = 0$) scenarios. Phenomenologically, in the first case the reflectivity variation is dominated by the increase of the QP-boson scattering, corresponding to a broadening of the Drude peak, whereas in the second case the decoupling between the QP and bosonic distributions can be rationalized in terms of a small increase of the plasma frequency without any change in the scattering rate. The difference between the two cases is more evident in the spectral region close to the dressed plasma frequency, $\Omega_p \approx 1$ eV—that is, an energy scale much higher than the energy scale of the bosonic function.

Time-resolved reflectivity measurements in the 0.5 to 2 eV photon-energy range (25) have been performed at $T = 300$ K on the same crystals (20) (OP96) used for the equilibrium optical spectroscopy. The $\delta R/R(\omega, t)$ two-dimensional matrix is reported in Fig. 2, A and B, along with the time traces at ~ 1.5 and 0.7 eV photon energies (white curves). After the pump excitation at $t = 0$, the temporal dynamics above and below Ω_p are very similar, exhibiting a relaxation dynamics of about 200 fs, generally attributed to the thermalization of electrons with SCPs (16), and a slower decay on the picosecond time scale, related to the thermalization with all other lattice vibrations. Shown in Fig. 2C are the energy-resolved traces at fixed delay ($t = 100$ fs). Comparing the $\delta R/R(\omega)$ measured on OP96 to the relative variation of the reflectivity calculated in the nonthermal and quasi-thermal cases (Fig. 1), we come to the major point of our work: The electrons thermalize with part of the bosonic excitations, described by $\Pi(\Omega)$, on a time scale faster than the electron-phonon thermalization. The fast time scale ($\ll 100$ fs) of this thermalization implies a very large coupling and a relatively small specific heat. These overall observations strongly suggest that this process involves bosonic excitations of electronic origin.

The relative strengths of $\Pi_b(\Omega)$ (where the index b refers to the three components in Eq. 1) determine (26) both the temporal evolution of the temperatures T_b , through the four-temperature model (4TM) (18), and the intensity of the reflectivity variation, through Eq. 2. As a consequence, the simultaneous fit of the calculated $\delta R/R(\omega, T_e, T_{be}, T_{SCP}, T_{lat})$ to the data reported in Fig. 2 in the time and frequency domain decisively narrows the phase space of the parameters of the model, as compared to single-color measurements. The fit procedure (18) allows

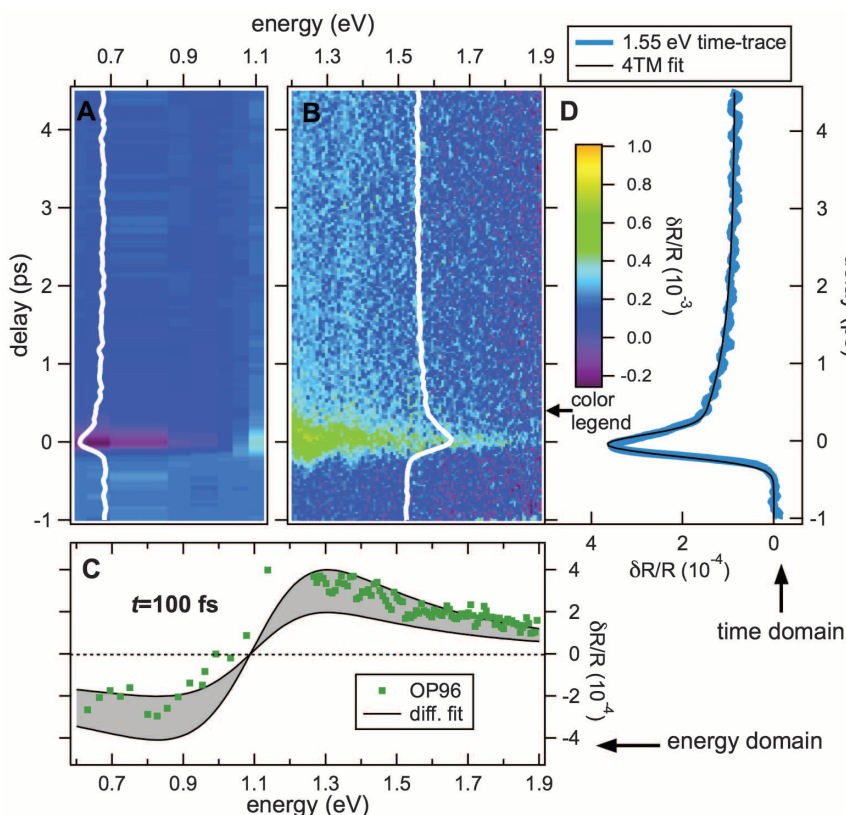
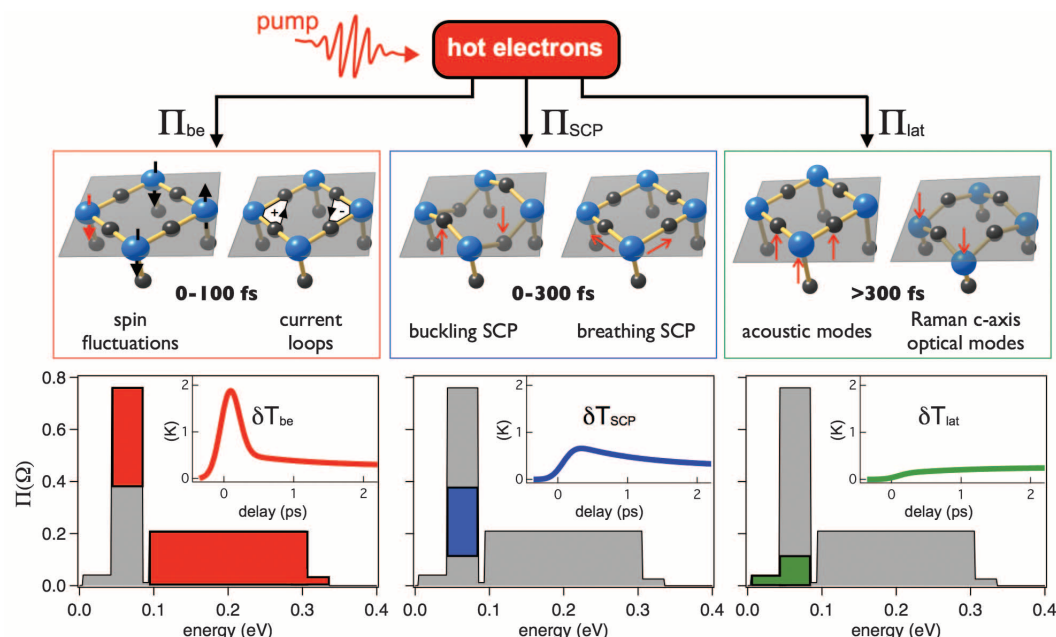


Fig. 2. Nonequilibrium optical spectroscopy. (A and B) Time- and frequency-resolved reflectivity measurements carried out on the OP96 sample at $T = 300$ K in the OPA (A) and supercontinuum (B) probe optical region (18). The white curves represent the time traces at photon energies of 1.55 eV and 0.65 eV, respectively. (C) Energy-resolved spectra for OP96, measured at fixed time $t = 100$ fs. The black lines are the maximum and minimum $\delta R/R(\omega, t = 100$ fs) determined through the fitting procedure described in the text and in (18) and accounting for the experimental uncertainty in the laser fluence and spot dimensions. Most of the values of $\delta R/R(t = 100$ fs) measured on OP96 at each wavelength fall between the two lines. (D) Time-resolved trace for OP96 at fixed photon energy of 1.55 eV. The black line is the best fit to the data obtained through the fitting procedure described in (18).

Fig. 3. Disentangling the contributions to the total bosonic function. The electronic (Π_{be} , red areas), strongly coupled phonon (Π_{SCP} , blue area), and lattice (Π_{lat} , green area) contributions to the total bosonic function are shown. The insets display the temporal evolution of the temperatures T_{be} (red line), T_{SCP} (blue line), and T_{lat} (green line) determined through the 4TM (18). Sketches of the possible microscopic mechanisms at the base of the different contributions to the total $\Pi(\Omega)$ are shown in the upper panels.



us to unambiguously extract the different contributions to $\Pi(\Omega)$ and to estimate C_{be} and C_{SCP} .

Figure 3 summarizes the main results of this work. The pump pulse of $10 \mu\text{J}/\text{cm}^2$ gently increases the electronic temperature by $\delta T_e \sim 2 \text{ K}$. The entire high-energy part and $\sim 46\%$ of the peak (red areas) instantaneously thermalize with electrons at a temperature $T_{be} \approx T_e$. The spectral distribution and the value of the specific heat of these excitations ($C_{be} < 0.1 C_e$) demonstrate their electronic origin. On a slower time scale (100 to 200 fs), the electrons thermalize with the SCPs that represent $\sim 20\%$ of the phonon density of states ($C_{SCP} = 0.2 C_{lat}$) but are responsible for $\sim 34\%$ of the coupling (blue area) in the peak of the bosonic function at 40 to 75 meV, corresponding to $\sim 17\%$ of the total bosonic function. Prominent candidates as SCPs are the buckling and breathing Cu-O optical modes. The third and last measured time scale is related to the thermalization with all other lattice modes (80% of the total), which include all acoustic modes and the infrared- and Raman-active modes involving c -axis motion of the Cu ions and provide less than 20% of the coupling (green area) in the peak of $\Pi(\Omega)$.

These results have important implications for the identification of the pairing mechanism in cuprates. The electron-boson coupling $\lambda_b = 2[\Pi_b(\Omega)/\Omega] d\Omega$ is calculated for each subset b of the bosonic excitations, considering the experimental uncertainties. In the strong-coupling regime ($\lambda_b < 1.5$), the critical temperature can be estimated (18) through an extended version of McMillan's equation (27), containing the logarithmic-averaged frequency ($\bar{\Omega}$) and the portion of $\Pi_b(\Omega)$ that contributes to the d-wave pairing (28). The maximal critical temperature attainable is calculated assuming that each $\Pi_b(\Omega)$ entirely contributes to the d-wave pairing. The

coupling with SCPs ($\lambda_{SCP} = 0.4 \pm 0.2$) is in complete agreement with the values measured on similar materials via different techniques, such as time-resolved photoemission spectroscopy (16), time-resolved electron diffraction (17), and single-color high-resolution time-resolved reflectivity (29). Although this value is rather close to the threshold of the strong-coupling regime (30, 31), the small value of $\bar{\Omega}$ gives $T_c = 2$ to 30 K, which is far from being able to account for the high-temperature superconductivity of the system. The coupling of electrons with all other lattice vibrations is even smaller in strength ($\lambda_{lat} = 0.2 \pm 0.2$) and provides an upper bound of the critical temperature of $T_c \approx 12 \text{ K}$. Finally, the large coupling constant ($\lambda_{be} = 1.1 \pm 0.2$) and the larger $\bar{\Omega}$ value of the electronic excitations give $T_c = 105$ to 135 K, and this alone accounts for the high critical temperature. We note that, whereas $\Pi_{SCP}(\Omega)$ and $\Pi_{lat}(\Omega)$ are expected to be temperature-independent, $\Pi_{be}(\Omega)$ increases as new magnetic excitations emerge when approaching T_c , particularly in the pseudogap phase (10). Therefore, the use of $\Pi_{be}(\Omega)$ determined at $T = 300 \text{ K}$ to estimate the QP-boson coupling and T_c underestimates the electronic contribution to the pairing, further supporting our conclusion of a dominant electronic mechanism in the superconductivity of cuprates. All the λ_b values, maximum attainable critical temperatures, and important parameters for each subset of the total bosonic function are reported in table S1.

The measured values of λ_{be} , λ_{SCP} , and λ_{lat} and the spectral distribution of the bosonic excitations strongly indicate that the antiferromagnetic spin fluctuations (7, 9) and the loop currents (3) are the most probable mediators for the formation of Cooper pairs. An isotope effect in the dispersion of nodal QPs has been observed by ARPES measurements on optimally doped Bi2212

(32). Analysis of these data (33) indicates that the nodal isotope effect can be explained by assuming that the QP-phonon coupling represents about 10% of the total contribution of other bosonic excitations. From Fig. 3, we estimate that the contribution of SCP is less than 20% of the total bosonic function. Hence, our results fully explain the observed isotope effect, providing a consistent interpretation of the most important experimental results about the QP-boson coupling in cuprates.

Our conclusions are rather independent of the assumption of the histogram-like form of $\Pi(\Omega)$ and are robust against modifications of the details of the equilibrium dielectric function. In fact, the outcome of this work strongly supports the factorization of the self-energy at $T = 300 \text{ K}$ into a temperature-dependent kernel function and the glue function $\Pi(\Omega)$ (see Eq. 2), even under non-equilibrium conditions. Although we do not exclude a priori that the upper limit used in the determination of the bosonic function can hide possible contributions to $\Pi(\Omega)$ even above 1 eV and that the electron-phonon coupling may cooperate in driving the superconducting phase transition, we demonstrate that bosonic excitations of electronic origin are the most important factor in the formation of the superconducting state at high temperatures in the cuprates. Our findings pave the way for the investigation of electron-boson coupling in a variety of complex materials, such as transition-metal oxides and iron-based superconductors.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/335/6076/1600/DC1
Materials and Methods
Fig. S1
Table S1
References (34, 35)

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Coherent Sensing of a Mechanical Resonator with a Single-Spin Qubit

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Mechanical systems can be influenced by a wide variety of small forces, ranging from gravitational to optical, electrical, and magnetic. When mechanical resonators are scaled down to nanometer-scale dimensions, these forces can be harnessed to enable coupling to individual quantum systems. We demonstrate that the coherent evolution of a single electronic spin associated with a nitrogen vacancy center in diamond can be coupled to the motion of a magnetized mechanical resonator. Coherent manipulation of the spin is used to sense driven and Brownian motion of the resonator under ambient conditions with a precision below 6 picometers. With future improvements, this technique could be used to detect mechanical zero-point fluctuations, realize strong spin-phonon coupling at a single quantum level, and implement quantum spin transducers.

Hybrid quantum systems offer many potential applications in quantum information science and quantum metrology. One example is cavity quantum electrodynamics (cQED), in which a quantum two-level system (a qubit) is strongly coupled to resonant photons in an electromagnetic cavity (1–3). In the mechanical analog of cQED, the qubit is coupled to a mechanical resonator, with resonant phonons playing the role of cavity photons (4–7). Mechanical resonators are promising components for hybrid quantum systems because they couple to a wide range of forces while maintaining high quality factors (8–13) and can be fabricated using scalable techniques. A recent experiment dem-

onstrated coupling of a superconducting phase qubit to the quantum motion of a piezoelectric resonator at millikelvin temperatures (11). However, this approach resulted in relatively short phonon lifetimes and is not easily extended to other resonator-qubit systems. An important goal is to extend this technique to nonpiezoelectric resonators that offer longer phonon lifetimes and to qubits featuring longer coherence times (4, 5, 9, 10, 14, 15). In particular, spin states of localized atomlike systems in the solid state can be manipulated individually and can serve as an exceptional quantum memory, even under ambient conditions. Moreover, experiments have demonstrated that resonators can be sensitive to the magnetic force associated with a single electronic spin (16). Control over a coupled spin-resonator hybrid quantum system could be used to mediate long-range spin-spin coupling for quantum information applications (17) or to manipulate mechanical motion at the single quantum level with long qubit and phonon coherence times (4, 11). In addition, coherent control over spin-phonon interactions is of direct relevance for realizations of novel nanoscale sensors (18–20).

In our experiment (Fig. 1A), we used the electronic spin associated with an individual nitrogen vacancy (NV) center to sense the motion of a nearby, magnetized atomic force microscopy (AFM) cantilever. The time-varying magnetic field generated by the displacement of the magnetic tip coherently drives the qubit evolution, which is subsequently detected via optical spin measurements. The essence of our approach (Fig. 1B) is to synchronize the evolution of the single spin with the cantilever's oscillations. When the spin is driven by a pulse train with a period matching the mechanical period, the motion constructively affects the spin evolution over a long interaction time, enhancing the spin's sensitivity in a narrow frequency band (21). In addition, the pulses dynamically decouple the NV spin from the slow evolution of the surrounding environment, increasing the possible interrogation time (22, 23).

The NV centers are implanted ~5 nm below the surface of a bulk diamond sample. The spin sublevels $|m_s = 0\rangle$ and $|m_s = \pm 1\rangle$ of the electronic ground state exhibit a zero-field splitting of ~2.87 GHz. In the presence of a static magnetic field, the degeneracy between $|+1\rangle$ and $|−1\rangle$ is lifted, allowing us to selectively address the $|0\rangle \rightarrow |+1\rangle$ transition with microwave radiation. The magnetic field $\mathbf{B}_{\text{tip}}$ generated by the tip at the position of the NV results in an additional Zeeman shift of the splitting, given by $\Delta\omega = g_e\mu_B \mathbf{B}_{\text{tip}} \cdot \hat{\mathbf{z}}/\hbar$, where $g_e \approx 2$ is the electron g-factor, μ_B is the Bohr magneton, $\hbar$ is Planck's constant divided by 2π , and $\hat{\mathbf{z}}$ is the unit vector along the NV axis. With the tip held at a constant position, the static Zeeman shift is detected by performing continuous wave (CW) electron spin resonance (ESR) measurements (Fig. 1C). By scanning the tip at a fixed height above the diamond surface with a piezoelectric scanning stage and monitoring $\Delta\omega$, we mapped the projection of $\mathbf{B}_{\text{tip}}$ along the NV axis (Fig. 1D). This provides an accurate measurement of the magnetic field gradient G_m produced by the tip along the NV axis, which

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reaches up to 10^5 T/m; see supporting online material for further details (24).

The gradient G_m translates mechanical oscillations of the cantilever $x_{ac}(t)$ into an oscillating magnetic field $B_{tip,ac}(t) = G_m x_{ac}(t)$. Coherent control over the NV spin is used to measure $B_{tip,ac}$ via pulsed ESR techniques such as spin echo and longer dynamical decoupling sequences (18, 22, 25). To probe Brownian motion, which has a characteristic frequency but a randomly varying amplitude and phase, our measurement sequence cannot be phase-locked to the mechanical oscillation. For this reason, we measure the variance of the accumulated phase, yielding the mean square field amplitude $\langle B_{tip,ac}^2 \rangle$. From $\langle B_{tip,ac}^2 \rangle$ and G_m , the mean square motional amplitude $\langle x^2 \rangle$ can be inferred, which, for an undriven resonator, is related to the thermal occupation number $\bar{n}_{th} = \frac{k\langle x^2 \rangle}{\hbar\omega_r}$ (where k is the spring constant and ω_r is the mechanical resonance frequency).

In the measurement sequence used to detect $\langle x^2 \rangle$ (Fig. 2A), the NV's electronic spin is polarized in the $|0\rangle$ state via green illumination (22, 26). Resonant microwaves tuned to the $|0\rangle \rightarrow |1\rangle$ transition are applied to perform a Hahn echo sequence with free precession periods of length τ . The final $\pi/2$ pulse of the sequence converts the relative accumulated phase into a population difference that is read out via fluorescence (Fig. 2B) (22). With the cantilever drive switched off, the data (blue triangles) demonstrate collapses and revivals in spin coherence due to nearby carbon-13 (^{13}C) nuclear spins undergoing Larmor precession at the frequency $\omega_{nuc} = \gamma_{nuc} B_{dc}$, where $\gamma_{nuc}/2\pi = 1.07$ kHz/G is the gyromagnetic ratio of the ^{13}C nuclei and B_{dc} is the magnitude of the static magnetic field (22, 26). The ensemble of ^{13}C nuclei produces an oscillating field with fluctuating amplitude and phase, resulting in collapses in coherence at odd multiples of π/ω_{nuc} and revivals at even multiples of π/ω_{nuc} (22). The tip is positioned relatively far from the NV, producing a weak gradient of $G_m = 100 \pm 12$ T/m, so in the absence of an external drive, the Brownian motion can be ignored and the field from the tip can be considered static. Red circles in Fig. 2B show the same measurement with the cantilever strongly driven. The evolution of the NV spin is dramatically affected by the cantilever motion. The revival is narrowed and shifted to $\tau = 2\pi/\omega_r$ due to $B_{tip,ac}$. Because the spin evolution is not phase-locked to the mechanical oscillation, $B_{tip,ac}$ has a constant amplitude but a uniformly distributed random initial phase ϕ_0 for each measurement, resulting in an echo signal given by a zero-order Bessel function (24). By fitting to this data, we determine the peak amplitude $B_{tip,ac} = 163 \pm 1$ mG and the oscillation frequency $\omega_r/2\pi = 80.14 \pm 0.07$ kHz, in agreement with the applied excitation frequency of 80.15 kHz. Incorporating the previously measured G_m , the root mean square (rms) amplitude of the tip motion, $x_{rms} = 114 \pm 14$ nm, can be extracted (24).

To measure the quality factor of the resonator (Fig. 2C), the resonator is driven to a fixed

amplitude of motion, the drive is switched off, and we subsequently measure x_{rms} as a function of wait time after turning off the cantilever drive using the same protocol as in Fig. 2B. A quality factor of $Q = 211 \pm 6$ is extracted from the exponential decay of x_{rms} , in agreement with measurements made independently, using conventional interferometric readout of the cantilever motion, that yield $Q = 221 \pm 4$.

Under ambient conditions, the $\omega_r/2\pi = 80.15$ kHz resonator with $k \approx 3$ N/m has a thermal occupation number of $\bar{n}_{th} \approx 8 \times 10^7$ phonons, corresponding to Brownian motion with an rms

amplitude of ~ 40 pm. To detect these oscillations with the single spin, we used a pulse sequence composed of N π -pulses known as XY4 (Fig. 3A) (23). This sequence further suppresses the effects of low-frequency magnetic noise and enhances the ac sensitivity by a factor of $\sim 1/(N^{3/2})$ in a narrow bandwidth (21, 23). It also has the advantage of self-correcting pulse errors to first order for any accumulated phase of the NV spin (23).

We used an XY4 sequence with $N = 12$ π -pulses to measure resonator motion at excitation amplitudes of $\sim 0, 57, 113$, and 170 pm of rms-driven motion (Fig. 3B). Unlike the measurements

Fig. 1. Concept of the experiment. (A) A magnetized AFM cantilever tip is positioned within tens of nanometers of a shallow implant NV in bulk diamond. (B) The control pulse sequence is matched to the period of the mechanical resonator to constructively accumulate phase from the oscillations. Green and blue shaded regions show the accumulated phase of the spin during one half-period; horizontal dashed arrows show the effect of the microwave π -pulses. (C) CW-ESR spectra showing the transitions between the $|m_s = 0\rangle$ and $|m_s = \pm 1\rangle$ ground states of the NV as the tip is moved parallel to the axis of cantilever oscillations. A 12-G external magnetic field is applied opposite the static field from the tip. The four traces correspond to ~ 100 -nm steps toward the NV. a.u., arbitrary units. (D) Scanning the tip at a fixed height above the surface, we extract a two-dimensional map of the dc field from the tip along the NV axis. Five pixels for which the shift could not be extracted were interpolated. (Inset) Fluorescence image of the diamond surface, with the NV used for this map circled in red.

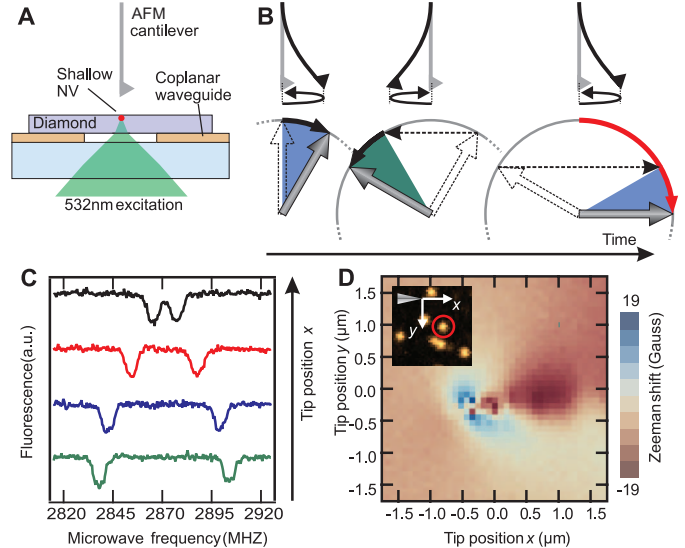
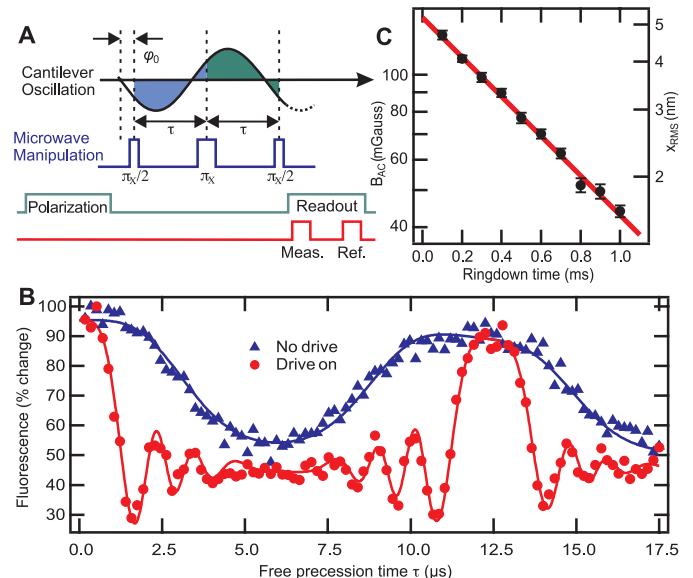


Fig. 2. Detection of driven motion (A) Hahn echo pulse sequence. The relative phase ϕ_0 varies for each measurement. (B) Spin-echo measurement with cantilever nearby with the drive switched off (blue triangles) and on (red circles). The Larmor precession of ^{13}C is tuned close to the mechanical frequency $\omega_r/2\pi = 80.15$ kHz via an external magnetic field of $B_{dc} \approx 78$ G. (C) Amplitude of ac magnetic field (left y axis) and motional amplitude (right y axis) as a function of wait time after switching off the drive, measured using spin echo. Here, $G_m = 2707 \pm 325$ T/m. Error bars correspond to the uncertainty in ac magnetic field (left y axis).



shown in Fig. 2, $B_{\text{ext,dc}}$ is tuned to a low value of ~ 10 G to extend the onset of the first collapse in spin coherence due to the ^{13}C nuclei's Larmor precession beyond the resonator-induced partial collapse (24). The multiple minima at 170 pm of rms-driven motion (green diamonds) are described by a Bessel function, as discussed above (24). Extracting x_{rms} from the fits in Fig. 3B and plotting against the ac driving voltage, the inclusion of Brownian motion is necessary to achieve

good agreement with the data (Fig. 3C). The impact of Brownian motion on the spin is also directly evident in Fig. 3B in the slight dip in the undriven data (red circles) at $\tau = \pi/\omega_r = 6.2$ μs .

To further enhance the impact of Brownian motion on our NV spin, we used an XY4 sequence with $N = 16$ π -pulses, allowing the spin to accumulate more motion-induced phase in a single measurement (Fig. 3D). With the tip nearby at a gradient of 2940 ± 265 T/m, the

Brownian motion induces a collapse in the NV spin coherence at $\tau = \pi/\omega_r$. The fits yield $x_{\text{rms}} = 40 \pm 5$ pm and $\omega_r/2\pi = 79.7 \pm 0.4$ kHz, in agreement with the expected value of ~ 40 pm and the resonance frequency of 80.15 kHz. This corresponds to $B_{\text{tip,ac}} = 117 \pm 9$ nT rms. The tip's proximity to the NV affects the spin coherence, resulting in the offset between the two curves.

Several recent experiments have reported that strongly driven mechanical resonators can influence the electronic spin of NV centers through the broadening and shifting of the spin transitions (15, 18, 19). Our approach takes advantage of the fact that the NV spin remains coherent for times much longer than the relatively short T_2^* (26). This longer interaction time enhances the spin's sensitivity to the resonator's motion (22, 25) and enables the detection of picometer-scale oscillations without phase-locking.

To understand the present sensitivity limits and the potential for improvement, we consider a measurement of an undriven cantilever as in Fig. 3D. When the pulse sequence is synchronized with the cantilever such that $\tau = \pi/\omega_r$, the probability P_1 to find the spin in state $|1\rangle$ after the sequence is $P_1 = \frac{1}{2} [1 + e^{-N(\pi/\omega_r T_2)^3}] - S$, where we isolated the signal S due to the cantilever motion (24)

$$S \approx \frac{1}{2} e^{-N(\pi/\omega_r T_2)^3} [1 - e^{-2N^2 \lambda^2 (2\pi n + 1) W / \omega_r^2}] \quad (1)$$

Here, the coupling rate $\lambda = g_e \mu_B G_m a_0 / \hbar$ is the Zeeman shift from a single phonon in the resonator, and $a_0 = \sqrt{\hbar/2m\omega_r}$ is the zero-point motion for a resonator of effective mass m . T_2 is the spin coherence time in the absence of the cantilever, whereas the exponent proportional to λ^2 describes the impact of the cantilever motion and depends on the mechanical Q , as well as the bandwidth of the control sequence through the factor $W = (1 + N/2Q)^{-1}$. The contribution from thermal motion is proportional to $\bar{n}_{\text{th}}$, and the remainder is due to quantum zero-point motion. Using Eq. 1 and including spin projection and shot noise through the single parameter K (24, 25), we obtain the phonon number sensitivity $\eta = \bar{n}_{\text{min}} \sqrt{t_{\text{tot}}} = \frac{\sqrt{\pi}}{2KW\lambda^2} e^{N(\pi/\omega_r T_2)^3} \left(\frac{\omega_r}{N}\right)^{3/2}$, where $\bar{n}_{\text{min}}$ is the minimum detectable phonon occupation number in total measurement time t_{tot} . For the experimental parameters of Fig. 3D, we obtain $\eta \approx 2.5 \times 10^8$ phonons/ $\sqrt{\text{Hz}}$, in agreement with the observed experimental sensitivity $\eta_{\text{exp}} \approx 4.9 \times 10^8$ phonons/ $\sqrt{\text{Hz}}$ (24).

The main factor currently limiting our sensitivity is the coupling strength λ . Though we measure gradients up to 10^5 T/m, which gives $\lambda/2\pi \approx 8$ Hz, our most sensitive measurements are taken at $\sim 3 \times 10^3$ T/m. The lower gradient is used because T_2 is found to decrease when the magnetic tip is very close to the NV; we observe a reduction of T_2 from ~ 160 μs when the tip is retracted to as low as ~ 5 μs when the tip is within 50 nm of the surface. Our use of XY4 dynamical decoupling sequences partially compensates for

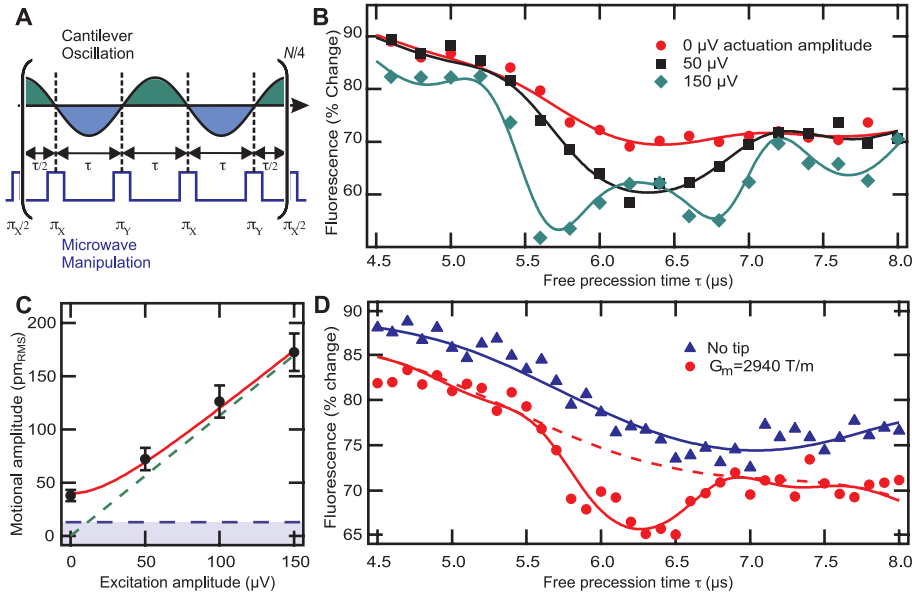


Fig. 3. Detection of Brownian motion. (A) XY4 pulse sequence. (B) XY4 measurement with $N = 12$ π -pulses for four different driving voltages (the 100- μV curve is excluded for clarity) at $G_m = 2978 \pm 268$ T/m. The fits account for Brownian and driven motion. (C) Extracted rms amplitude from (B) as a function of driving voltage. The red curve shows the expected rms amplitude based on independent calibrations, including thermal and driven motion, whereas the green dashed line is expected from driven motion alone. Neither line has any free parameters. The blue dashed line shows the estimated minimum detectable motion for the measurements in (B). Error bars indicate the statistical uncertainty in the fits and field-gradient measurements used to extract the motional amplitude. (D) XY4 measurement with $N = 16$ π -pulses, with the tip nearby at $G_m = 2940 \pm 265$ T/m (red circles) and without the tip (blue triangles). Solid lines are fits. The dashed red line shows the expected signal in the presence of a static tip with no Brownian motion.

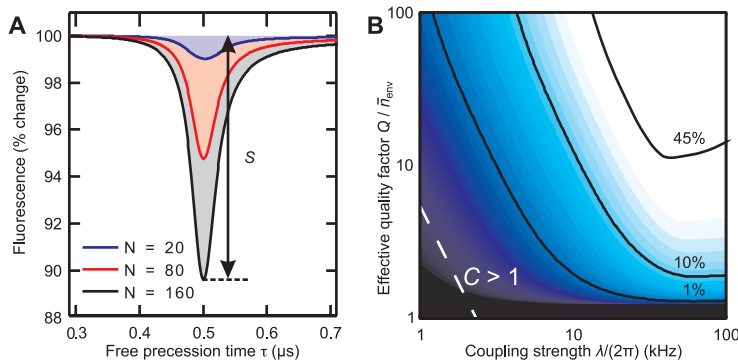


Fig. 4. Prospects for detection of zero-point motion. (A) Calculated XY4 signal for a cantilever cooled near its ground state, starting from an environmental temperature of $T_{\text{env}} = 4$ K. The signal S is the measurable impact from cantilever motion. Parameters are $\omega_r/2\pi = 1$ MHz, $\lambda/2\pi = 10$ kHz, $T_2 = 1$ ms, and $Q = 10^6$. (B) Signal S versus coupling strength and effective quality factor. The white dashed line marks the threshold of strong cooperativity, $C = \lambda^2 Q_{\text{eff}} T_2 / \omega_r > 1$. At each point, we optimize the pulse number N within the experimental limitation of $N_{\text{max}} \leq 160$, as shown to be feasible in recent experiments (21, 23). The slight upturn of the contours at large coupling is due to cantilever-induced decoherence (24); this small correction is neglected in Eq. 1.

this effect, indicating that the reduction in T_2 is due to low-frequency noise, which we attribute to magnetic domain noise of the tip material. We expect that this effect can be mitigated using alternative tip materials such as rare earth ferromagnets (27). The coupling strength can be further improved by using customized nanoresonators with a larger zero-point motion. Using state-of-the-art nanofabrication techniques, resonators with $Q > 10^6$, $\omega_r/2\pi \sim 1$ MHz (28), $G_m \sim 10^5$ T/m, and a coupling strength of $\lambda/2\pi \sim 10$ kHz can be fabricated (4), which, together with extended coherence times in isotopically purified diamond of $T_2 > 2$ ms (29) and faster pulse sequences with $N \sim 136$ pulses (23), yield a projected sensitivity of $\eta < 1$ phonon/ $\sqrt{\text{Hz}}$. Combined with recently demonstrated single-shot spin readout (30), this raises the intriguing prospect of using a single NV center to sense mechanical motion at the scale of zero-point fluctuations in a single shot.

To assess the feasibility of sensing zero-point motion (Fig. 4A), we assume that the resonator is actively cooled near its motional ground state, using either the spin (4) or an additional system such as an optical or microwave cavity (12, 13). Such cooling schemes are always accompanied by a decreased effective mechanical quality factor, $Q_{\text{eff}} = Q/\bar{n}_{\text{env}}$, where $\bar{n}_{\text{env}}$ is the phonon occupation number at the temperature of the surrounding environment. Inserting $\bar{n}_{\text{th}} = 0$ and $W = 2Q_{\text{eff}}/N$ into Eq. 1, a near maximal signal $S \sim 1/2$ is obtained, provided that $C = \lambda^2 Q_{\text{eff}} \bar{T}_2 / \omega_r > 1$, where $\bar{T}_2 = T_2 N^{2/3}$ is the extended spin-coherence time due to dynamical decoupling (23). This is verified in Fig. 4B, which demonstrates that for a wide range of realistic parameters with $C > 1$, the zero-point motion of the resonator results in $S \sim 1/2$. The parameter C is a fundamental quantity in the physics of spin-phonon interactions. In direct analogy to the so-called cooperativity in cQED, $C > 1$ marks the onset of coherent quantum effects in a coupled spin-phonon system. Taking the optimized but realistic values $\lambda/2\pi = 10$ kHz, $T_2 = 1$ ms, $Q = 10^6$, $\omega_r/2\pi = 1$ MHz, and $N = 160$ pulses, we find that $C \sim 35$ can be reached at an environmental temperature of $T = 4$ K. Besides detection of zero-point motion, entering this regime could also enable coherent, long-range interactions between individual spins mediated by a mechanical resonator (17), which are of great interest for developing scalable, spin-based quantum information systems. Furthermore, by operating at lower environmental temperatures of $T \sim 100$ mK, it becomes possible to use the spin to cool the resonator down to its ground state (4) and to engineer quantum superpositions of mechanical motion, which could be read out using a coherent detection scheme like the one we present in this work.

The above considerations indicate that our approach provides an experimentally feasible route toward reaching strong coupling between single phonons and spins. Potential applications ranging from the creation and detection of quantum states of mechanical motion and the realization of quantum

spin transducers to novel approaches for nano-scale sensing and readout (19, 20) can be foreseen.

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Materials and Methods

SOM Text

Figs. S1 to S3

Tables S1 and S2

References

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Hydrocarbon Separations in a Metal-Organic Framework with Open Iron(II) Coordination Sites

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The energy costs associated with large-scale industrial separation of light hydrocarbons by cryogenic distillation could potentially be lowered through development of selective solid adsorbents that operate at higher temperatures. Here, the metal-organic framework $\text{Fe}_2(\text{dobdc})$ (dobdc^{4-} : 2,5-dioxido-1,4-benzenedicarboxylate) is demonstrated to exhibit excellent performance characteristics for separation of ethylene/ethane and propylene/propane mixtures at 318 kelvin. Breakthrough data obtained for these mixtures provide experimental validation of simulations, which in turn predict high selectivities and capacities of this material for the fractionation of methane/ethane/ethylene/acetylene mixtures, removal of acetylene impurities from ethylene, and membrane-based olefin/paraffin separations. Neutron powder diffraction data confirm a side-on coordination of acetylene, ethylene, and propylene at the iron(II) centers, while also providing solid-state structural characterization of the much weaker interactions of ethane and propane with the metal.

As a consequence of the similar sizes and volatilities of the molecules, separations of olefin/paraffin mixtures, such as ethylene/ethane and propylene/propane, must currently be performed at low temperatures and high pressures and are among the most energy-intensive separations carried out at large scale in the chemical industry (1). Because these gas mix-

tures are produced by cracking long-chain hydrocarbons at elevated temperatures, a substantial energy penalty arises from cooling the gases to the low temperatures required for distillation. Thus, tremendous energy savings could be realized if materials enabling the efficient separation of olefins and paraffins at higher temperatures (than currently used in distillation) and atmo-

spheric pressure were achieved. Competing approaches toward this end include membrane designs (2) and organic solvent-based sorbents (3), as well as porous solid adsorbents featuring selective chemical interactions with the carbon-carbon double bond in olefins. In this latter category, metal-organic frameworks (MOFs), which offer high surface areas, adjustable pore dimensions, and chemical tunability, have received considerable attention as adsorbents in gas storage and gas separation applications, with particular emphasis on the dense storage of methane (4, 5) and hydrogen (6, 7) and on the efficient removal of carbon dioxide from flue gas (8) and natural gas deposits (9, 10). More recently, the potential utility of these porous structures for the separation of hydrocarbon mixtures has been exposed (11–15), specifically for the separation of ethylene/ethane and propylene/propane mixtures. Herein, we show that $\text{Fe}_2(\text{dobdc})$, a metal-organic framework with exposed iron(II) coordination sites exhibiting high olefin/paraffin selectivities, can be used for the separation of ethylene/ethane and propylene/propane. Furthermore, simulations that are validated by the experimental work presented here allow us to predict that this framework may be further capable of removing acetylene from the ethylene produced by a naphtha cracker and

fractionating a methane/ethane/ethylene/acetylene mixture into its pure components. These simulations further predict that $\text{Fe}_2(\text{dobdc})$ exhibits higher adsorption selectivities and hydrocarbon separation capacity than a number of recently reported solid adsorbents.

The redox-active metal-organic framework $\text{Fe}_2(\text{dobdc})$ (dobdc^{4-} : 2,5-dioxido-1,4-benzenedicarboxylate), also referred to as Fe-MOF-74 or CPO-27-Fe, was selected for testing in separating olefin/paraffin mixtures owing to its previously demonstrated ability to bind O_2 at the iron centers in a side-on manner (16). This framework displays a Brunauer-Emmett-Teller surface area of $1350 \text{ m}^2/\text{g}$ and $11 \text{ \AA}$ -wide channels lined with square pyramidal Fe^{2+} cations, each with an open coordination site accessible to incoming adsorbate molecules (Fig. 1). With a compact tetra-anionic bridging ligand, the structure features an extremely high surface density of $2.9 \text{ Fe}^{\text{II}}$ coordination sites available per $100 \text{ \AA}^2$ on its surface, with spacings of just $6.84(1)$ and $8.98(2) \text{ \AA}$ between iron atoms along and around a channel, respectively. Thus, it appears to provide a near-optimal platform for the high-capacity adsorption of small olefins, such as ethylene and propylene. Furthermore, the Mg^{2+} or Co^{2+} analogs of this structure type have recently been shown to display selective adsorption for olefins over paraffins (17, 18). The higher surface area and softer metal character of $\text{Fe}_2(\text{dobdc})$ as compared to the recently reported materials should lend both higher selectivity and capacity to the iron(II) framework.

To investigate the ability of $\text{Fe}_2(\text{dobdc})$ to adsorb light hydrocarbons, pure component equilibrium adsorption isotherms for methane, ethane, ethylene, acetylene, propane, and propylene were measured at 318, 333, and 353 K. Figure 2 shows the data obtained at 318 K, with the remaining

data presented in fig. S1. As evidenced by the initial steep rise in the isotherms, $\text{Fe}_2(\text{dobdc})$ displays a strong affinity for the unsaturated hydrocarbons acetylene, ethylene, and propylene. Additionally, the uptake of these gases at 1 bar approaches the stoichiometric quantity expected if one gas molecule is adsorbed per iron(II) center. The propane and ethane adsorption capacities under these conditions, although lower than those of their unsaturated counterparts, are both considerably higher than observed for methane, which has a lower polarizability and a smaller kinetic diameter. Importantly, all of the isotherms are completely reversible and exhibit no hysteresis. Further equilibrium adsorption experiments at 318 K (fig. S2) indicate no loss in olefin uptake capacity over 15 ethylene adsorption/desorption cycles. Additionally, no loss in propylene uptake was observed after 40 adsorption/desorption cycles, as verified by thermogravimetric analysis (fig. S2).

Neutron powder diffraction experiments were carried out to determine the nature of the interactions of these adsorbate molecules within $\text{Fe}_2(\text{dobdc})$. In a typical experiment, $\text{Fe}_2(\text{dobdc})$ was dosed with deuterated gas at 300 K and cooled to 4 K for data collection. Rietveld refinements were performed against these data to provide the structural models presented in Fig. 1. We recently employed this technique to investigate the coordination of dioxygen to the iron centers of this material (16). Analogous to these previous results, only one adsorption site is apparent, corresponding to the open coordination site of the exposed Fe^{2+} cations, upon dosing substoichiometric equivalents of gas per framework iron. The unsaturated hydrocarbons acetylene, ethylene, and propylene indeed display the anticipated side-on binding modes, with Fe-C distances lying in the range $2.42(2)$ to $2.60(2) \text{ \AA}$. These distances are sub-

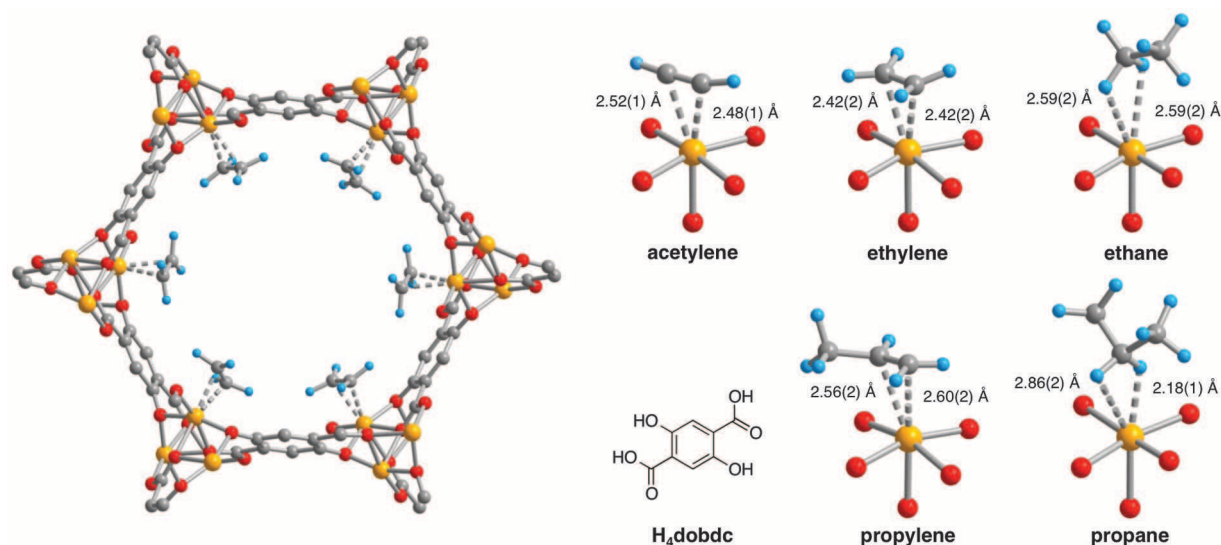


Fig. 1. (Left) A portion of the solid-state structure of $\text{Fe}_2(\text{dobdc}) \cdot 2\text{C}_2\text{D}_4$ as determined by analysis of neutron powder diffraction data; orange, red, gray, and blue spheres represent Fe, O, C, and D atoms, respectively. The view is along the [001] direction and shows an ethylene molecule bound to the open coordination site at each iron(II) center. (Right) H_4dobdc ligand and the first coordination

spheres for the iron centers in the solid-state structures obtained upon dosing $\text{Fe}_2(\text{dobdc})$ with acetylene, ethylene, ethane, propylene, and propane. For propane in $\text{Fe}_2(\text{dobdc})$, the adsorbed hydrocarbon molecule has orientational disorder with respect to the open metal center. Of several refined models, the single molecule with large displacement parameters is the most reasonable.

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stantially longer than the separations of 2.020(5) to 2.078(4) Å observed for the diamagnetic complex $[\text{Fe}(\text{C}_2\text{H}_4)_4]^{2-}$, one of the very few iron(II)-olefin species to be structurally characterized previously (19). The difference suggests that the metal centers within $\text{Fe}_2(\text{dobdc})$ maintain a high-spin electron configuration when binding these gases, consistent with weaker interactions that can be reversed with little energy penalty. The interactions of both ethane and propane with the metal cations in $\text{Fe}_2(\text{dobdc})$ are even weaker, as evidenced by the elongated Fe-C distance of ~3 Å. This is in good agreement with the Mg-C distance reported for methane adsorption in $\text{Mg}_2(\text{dobdc})$, a system in which the metal-adsorbate interactions are also a result of ion-induced dipole interactions between coordinatively unsaturated metal cations and a hydrocarbon (20).

Variable-temperature magnetic susceptibility measurements were performed to probe the electronic state of the iron centers in $\text{Fe}_2(\text{dobdc})$ upon gas binding. As shown in Fig. 3, $\text{Fe}_2(\text{dobdc})$ itself displays a $\chi_M T$ value of 6.40 $\text{cm}^3\text{K/mol}$ at 300 K, which gradually rises as the temperature decreases before turning over and dropping sharply below 28 K. The behavior is consistent with the presence of high-spin ($S = 2$) iron(II) centers exhibiting

weak [$J = 4.1(1) \text{ cm}^{-1}$] ferromagnetic coupling along the oxo-bridged chains running parallel to the c axis, together with still weaker antiferromagnetic coupling between chains. Under a pressure of 1 bar of methane, ethane, ethylene, acetylene, propane, or propylene, the high-spin electron configuration of the iron(II) centers is maintained, but the nature of the magnetic exchange along the chains is altered to varying extents. Weakly interacting adsorbates, such as methane, ethane, and propane, slightly diminish the strength of the ferromagnetic exchange, whereas the more strongly interacting propylene, ethylene, and acetylene perturb the electron density at the iron(II) centers sufficiently to reverse the nature of the intrachain coupling from ferromagnetic to antiferromagnetic. Moreover, the J values resulting from the fits to the data provide a qualitative measure of the adsorbate binding energy, suggesting that the strength of the iron(II)-hydrocarbon interactions increase along the series methane < ethane < propane < propylene < acetylene < ethylene.

The strength of hydrocarbon binding within $\text{Fe}_2(\text{dobdc})$ was determined quantitatively through analysis of the gas adsorption data. The data for acetylene, ethylene, ethane, propane, and propylene, expressed in terms of absolute loadings, were

fitted with the dual-Langmuir-Freundlich isotherm model, whereas methane adsorption data were fitted with a single-site Langmuir model (21). Isothermic heats of adsorption were then calculated from the fits to compare the binding enthalpies of these gases under various loadings (fig. S3). Heats of adsorption for acetylene (−47 kJ/mol), ethylene (−45 kJ/mol), and propylene (−44 kJ/mol) show a significant reduction as the loading approaches the value corresponding to one gas molecule per iron(II) center, again consistent with the exposed metal cations presenting the strongest adsorption sites in the material. Propane (−33 kJ/mol), ethane (−25 kJ/mol), and methane (−20 kJ/mol) adsorption enthalpies are all considerably lower in magnitude, with the trend reflecting the decreasing polarizabilities of these molecules from propane to ethane to methane.

Adsorption selectivities were calculated using ideal adsorbed solution theory (IAST) (22), using the fitted isotherms of the experimental isotherm data for relevant gas mixtures in $\text{Fe}_2(\text{dobdc})$ and a number of other porous materials for which analogous gas uptake properties have been reported (fig. S4). For an equimolar mixture of ethylene and ethane at 318 K, the adsorption selectivities obtained for $\text{Fe}_2(\text{dobdc})$ of 13 to 18 are significantly greater than those calculated for either zeolite NaX or the isostructural metal-organic framework $\text{Mg}_2(\text{dobdc})$, which display selectivities of 9 to 14 and 4 to 7, respectively (17, 23). The latter result is consistent with the softer character of Fe^{2+} relative to Mg^{2+} , leading to a stronger interaction with the π electron cloud of the olefin. Similarly, in comparing the performance of $\text{Fe}_2(\text{dobdc})$ with other porous materials for the separation of a propane/propylene mixture (selectivity = 13 to 15), it is rivaled in selectivity only by zeolite ITQ-12, which displays adsorption selectivity of 15 while the other materials display selectivities from 3 to 9 (24). However,

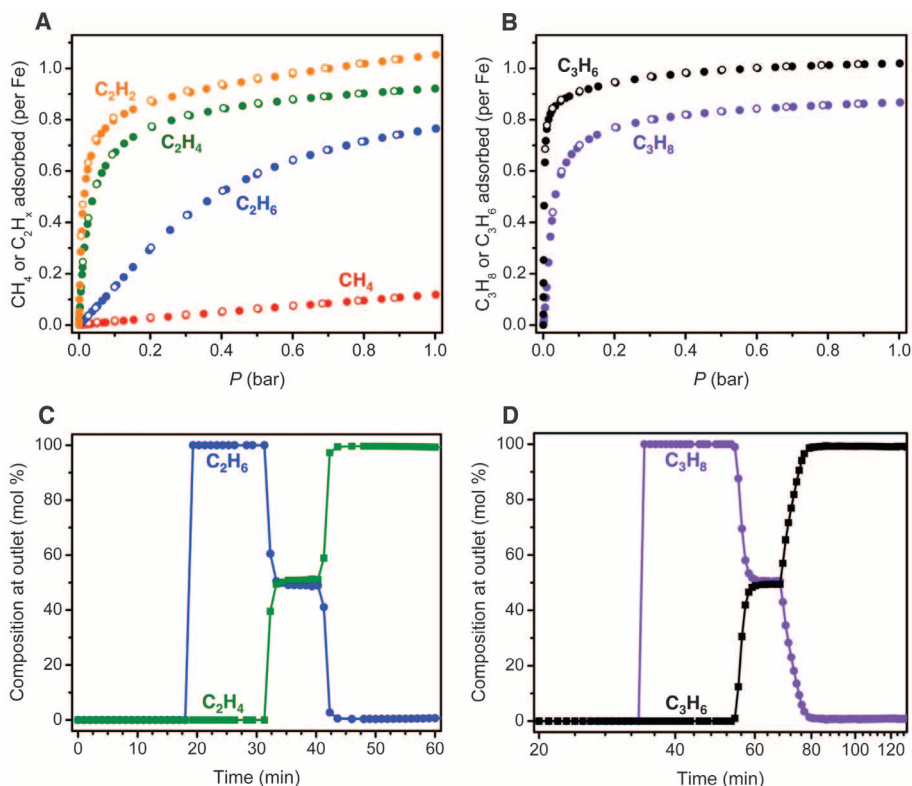


Fig. 2. (A and B) Gas adsorption isotherms for (A) methane, ethane, ethylene, and acetylene and (B) propane and propylene in $\text{Fe}_2(\text{dobdc})$ at 318 K. Filled and open circles represent adsorption and desorption data, respectively. The adsorption capacities at 1 bar correspond to 0.77, 5.00, 6.02, 6.89, 5.67, and 6.66 mmol/g, respectively. (C and D) Experimental breakthrough curves for the adsorption of equimolar (C) ethane/ethylene and (D) propane/propylene mixtures flowing through a 1.5-mL bed of $\text{Fe}_2(\text{dobdc})$ at 318 K with a total gas flow of 2 mL/minute at atmospheric pressure. After breakthrough of the olefin and return to an equimolar mixture composition, a nitrogen purge was applied, leading to desorption of the olefin. In an actual separation scenario, desorption would instead be carried out by applying a vacuum and/or raising the temperature.

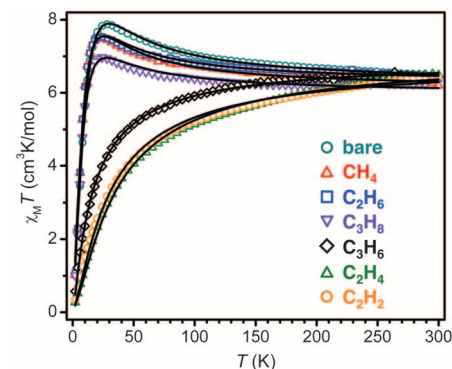


Fig. 3. Variable-temperature magnetic susceptibility data in an applied field of 1 kOe for samples of $\text{Fe}_2(\text{dobdc})$ in a vacuum (bare) and under 1 bar of the indicated hydrocarbon. Black lines represent fits to a Hamiltonian derived using the Fisher model for a one-dimensional chain of exchange-coupled $S = 2$ ions, with an additional term to account for interchain coupling. Full details of the model and fit parameters can be found in table S17 and accompanying text.

the selectivities of ITQ-12 for this mixture were calculated from data collected at 303 K, and it is expected that the selectivity of this material will be lower at higher temperatures. Adsorption selectivities were also calculated using IAST for $\text{Fe}_2(\text{dobdc})$ in an equimolar four-component mixture of methane, ethane, ethylene, and acetylene at 318 K, as relating to the purification of natural gas. For an adsorption-based process operating at 1 bar, the calculated acetylene/methane, ethylene/methane, and ethane/methane selectivities are 700, 300, and 20, respectively. These values are much higher than those recently reported (13.8, 11.1, and 16.6, respectively) for a zinc-based metal-organic framework, also based on an analogous calculation procedure (25).

To evaluate the performance of $\text{Fe}_2(\text{dobdc})$ in an actual adsorption-based separation process, breakthrough experiments were performed in which an equimolar ethylene/ethane or propylene/propane mixture was flowed over a packed bed of the solid with a total flow of 2 mL per minute at 318 K (Fig. 2). In a typical experiment, the gas mixture was flowed through 300 to 400 mg of metal-organic framework crystallites packed into a 1.5-mL glass column, and the outlet gas stream was monitored by a gas chromatograph equipped with a flame ionization detector. As expected from the calculated selectivities, in each case, the alkane was first to elute through the bed, whereas the solid adsorbent retained the olefin. For the C_3 hydrocarbons, the outlet gas contained undetectable levels of propylene, resulting in a propane feed that appeared to be 100% pure, within the detection limit of the instrument [~ 100 parts per million (ppm)]. Upon saturation of the metal centers within the adsorbent, propylene “broke through,” and the outlet gas stream then quickly reached equimolar concentrations. By stopping the gas feed and flowing a purge of nitrogen through the bed, the small amount of weakly bound propane remaining in the pores of the framework could be quickly removed, and the iron-bound

propylene then desorbed more slowly. Greater than 99% pure propylene was realized during the desorption step of the breakthrough experiment. In a similar manner, breakthrough experiments showed that $\text{Fe}_2(\text{dobdc})$ can separate an equimolar mixture of ethylene and ethane into the pure component gases of 99% to 99.5% purity. Differential scanning calorimetry (DSC) experiments (fig. S5) indicate that energies of 0.84 MJ/kg and 1.3 MJ/kg are needed to release propylene and ethylene, respectively, and regenerate the material for subsequent separation steps.

Although breakthrough experiments are quite valuable for evaluating the gas separation capabilities of a material, in practice they can be difficult and time consuming. In order to compare $\text{Fe}_2(\text{dobdc})$ with other reported adsorbents for ethylene/ethane and propylene/propane separations, we sought to demonstrate that the breakthrough characteristics could instead be simulated with reasonable accuracy. Assuming that (i) intracrystalline diffusion is negligible through an isothermal adsorption bed in thermodynamic equilibrium, (ii) plug flow proceeds through the bed, and (iii) the binary mixture adsorption equilibrium in the packed bed of crystallites can be calculated using IAST, we were able to solve a set of partial differential equations and calculate breakthrough curves for both ethylene/ethane and propylene/propane mixtures. The resulting transient gas composition profiles (figs. S6 and S7) are in excellent agreement with the experimental results shown in Fig. 1.

Given this validation, we employed analogous simulations to make quantitative comparisons with other materials. From the simulated breakthrough curves, the time interval during which the exit gas compositions have a purity of 99% propane can be determined, together with the amount of 99% pure propane produced in this time interval. The production capacities, expressed as the amount of propane produced per liter of adsorbent, are shown in fig. S8 over a range of pressures for the zeolites

ITQ-12 at 303 K and NaX at 318 K, and for the metal-organic frameworks $\text{Cu}_3(\text{btc})_2$ (btc^{3-} : 1,3,5-benzenetricarboxylate) at 318 K (26), $\text{Cr}_3(\text{btc})_2$ (27) at 308 K (fig. S9), and Fe-MIL-100 at 303 K (11). These results indicate that the propane production capacity of $\text{Fe}_2(\text{dobdc})$ at 318 K, which ranges up to 5.8 mol/L at a total pressure of 1.0 bar, is at least 20% higher than that of any of these other materials. A similar method was used to calculate the amount of polymer-grade (99.5%+) propylene that can be produced by these materials, again leading to a higher capacity for $\text{Fe}_2(\text{dobdc})$ than for any other material. The compound $\text{Mg}_2(\text{dobdc})$ exhibits a lower productivity than $\text{Fe}_2(\text{dobdc})$, a result of the lower adsorption selectivity of this material. Although zeolite ITQ-12 displayed a comparable selectivity to $\text{Fe}_2(\text{dobdc})$, its capacity limitation, which stems from its low pore volume of 0.134 cm^3/g , results in a propylene productivity that is just 47% of that of the metal-organic framework. For the separation of ethylene/ethane mixtures, the breakthrough simulations indicate an even greater advantage of $\text{Fe}_2(\text{dobdc})$ over other adsorbents, with production capacities that are roughly double those of $\text{Mg}_2(\text{dobdc})$ and zeolite NaX (fig. S10).

In addition to the separation of binary olefin/paraffin mixtures, there is tremendous current interest in separating ethane, ethylene, and acetylene from methane for the purification of natural gas. Indeed, a number of porous materials (23, 28) are able to selectively separate methane from mixtures including C_2 hydrocarbons (ethane, ethylene, and acetylene). These materials, however, are unable to simultaneously purify the ethane, ethylene, and acetylene being removed from the gas stream. A separation process that uses the same adsorptive material for the separation and purification of all four components of a C_1/C_2 mixture could potentially lead to substantial efficiency and energy savings over current processes. To establish the feasibility of using $\text{Fe}_2(\text{dobdc})$ for this task, we carried out breakthrough calculations for such a mixture. Figure 4 presents simulated data on the gas-phase molar concentrations exiting an adsorber packed with $\text{Fe}_2(\text{dobdc})$ and subjected to a feed gas consisting of an equimolar mixture of methane, ethane, ethylene, and acetylene at a total pressure of 1 bar and a temperature of 318 K. The breakthrough times reflect the relative adsorption selectivities (acetylene > ethylene > ethane > methane) for the material, and the curves indicate a clean, sharp breakthrough transition for each successive gas.

Based on these results, the diagram at the right in Fig. 4 demonstrates how it might be possible to procure pure methane, ethane, ethylene, and acetylene using three packed beds of $\text{Fe}_2(\text{dobdc})$. In this process, a gas mixture is fed into the first bed, and methane—the fraction with the lowest adsorptivity—breaks through first. Pure methane can be collected until the second gas, ethane, breaks through. When the third component of the gas stream, ethylene, is present in the

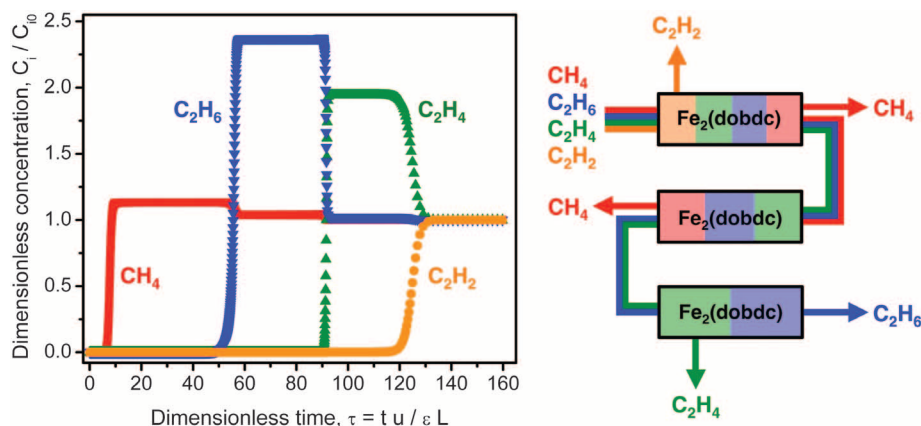


Fig. 4. (Left) Calculated methane (red), ethane (blue), ethylene (green), and acetylene (orange) breakthrough curves for an equimolar mixture of the gases at 1 bar flowing through a fixed bed of $\text{Fe}_2(\text{dobdc})$ at 318 K. (Right) Schematic representation of the separation of a mixture of methane, ethane, ethylene, and acetylene using just three packed beds of $\text{Fe}_2(\text{dobdc})$ in a vacuum swing adsorption or temperature swing adsorption process.

eluent, the gas flow is diverted to a second bed, from which additional pure methane is collected during the adsorption step and from which a mixture of ethane and ethylene is subsequently desorbed. This ethane/ethylene mixture is then separated into its pure components using a third adsorbent bed. By halting the feed into the first bed just before the breakthrough of acetylene, pure acetylene can be obtained via desorption.

Ethylene produced in a naphtha cracker contains an impurity of approximately 1% acetylene. However, there are strict limitations to the amount of acetylene that can be tolerated in the feed to an ethylene polymerization reactor. The current technology for this purpose uses absorption with liquid *N,N'*-dimethylformamide, but the use of solid adsorbents could potentially provide an energy-efficient alternative. We therefore also investigated the use of $\text{Fe}_2(\text{dobdc})$ for removal of acetylene from mixtures with ethylene. Simulated breakthrough characteristics for a feed mixture containing 1 bar of ethylene and 0.01 bar of acetylene at 318 K indicate that final acetylene concentrations on the order of 10 ppm could be realized (fig. S11).

An alternative to the use of pressure swing adsorption for olefin/paraffin separations is to adopt a membrane-based technology. To investigate the potential use of $\text{Fe}_2(\text{dobdc})$ membranes for the separation of such mixtures, we applied a simulation methodology previously employed for evaluating idealized $\text{Mg}_2(\text{dobdc})$ membranes (29, 30). Here, an equimolar ethylene/ethane or propylene/propane mixture permeates through a contiguous, unsupported layer of $\text{Fe}_2(\text{dobdc})$ crystals, all aligned such that the channels of the framework are oriented parallel to the gas flow. Although a membrane of this type would be challenging to prepare, the following results indicate the utility of an unsupported metal-organic framework membrane. The permeation fluxes for the gas components are then related to the gradients in the loadings within the crystals by the Maxwell-Stefan diffusion equations (29–31). The calculated ethylene/ethane permeation selectivities lie in the range of 13 to 20 for total upstream pressures between 0.1 and 1.0 bar (fig. S12). These values are close to the corresponding adsorption selectivities in $\text{Fe}_2(\text{dobdc})$ because the more mobile partner species are slowed down within the one-dimensional channels of the structure. The slowing-down effects are caused by correlations in the molecular hops of the mobile and tardier species in the mixture within the one-dimensional channels of structures such as $\text{Fe}_2(\text{dobdc})$ and $\text{Mg}_2(\text{dobdc})$ (31). Such correlations serve to bring the component diffusivities in the mixture closer to each other.

In other words, strong correlation effects within the one-dimensional channels of $\text{Fe}_2(\text{dobdc})$ cause the permeation selectivities to be close to the adsorption selectivities, which, as discussed above, are very high. The calculated permeation selectivities are expected to be reasonably accurate, irrespective of the accuracy of the force field information, because the high degree of correla-

tions within the channels tends to eliminate differences in the component mobilities. Thus, much greater selectivity can be expected for membranes based on $\text{Fe}_2(\text{dobdc})$ compared to ZIF-8, for which a permeation selectivity of 2.8 was recently reported (32). An important further advantage of the use of $\text{Fe}_2(\text{dobdc})$ is that the diffusivities within the 11 Å-wide channels of this material are about two to three orders of magnitude greater than those in ZIF-8, conferring both selectivity and permeability advantages. Similar advantages can be expected for applications of $\text{Fe}_2(\text{dobdc})$ in membrane separations of equimolar propylene/propane mixtures, for which permeation selectivities are calculated to lie in the range of 14 to 16 for total upstream pressures between 0.05 and 1.0 bar (fig. S13).

The foregoing results demonstrate the extraordinary prospects for using the metal-organic framework $\text{Fe}_2(\text{dobdc})$ as a solid adsorbent in the separation of valuable C_1 to C_3 hydrocarbons through pressure/temperature swing adsorption methods or through membrane-based applications.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/335/6076/1606/DC1
Materials and Methods
Figures S1 to S13
Tables S1 to S23
Movies S1 to S11
References (33–45)

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$^{238}\text{U}/^{235}\text{U}$ Systematics in Terrestrial Uranium-Bearing Minerals

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The present-day $^{238}\text{U}/^{235}\text{U}$ ratio has fundamental implications for uranium-lead geochronology and cosmochronology. A value of 137.88 has previously been considered invariant and has been used without uncertainty to calculate terrestrial mineral ages. We report high-precision $^{238}\text{U}/^{235}\text{U}$ measurements for a suite of uranium-bearing minerals from 58 samples representing a diverse range of lithologies. This data set exhibits a range in $^{238}\text{U}/^{235}\text{U}$ values of >5 per mil, with no clear relation to any petrogenetic, secular, or regional trends. Variation between comagmatic minerals suggests that $^{238}\text{U}/^{235}\text{U}$ fractionation processes operate at magmatic temperatures. A mean $^{238}\text{U}/^{235}\text{U}$ value of 137.818 ± 0.045 (2 σ) in zircon samples reflects the average uranium isotopic composition and variability of terrestrial zircon. This distribution is broadly representative of the average crustal and “bulk Earth” $^{238}\text{U}/^{235}\text{U}$ composition.

The uranium-lead (U-Pb) system is widely used as an isotopic chronometer for geological and meteoritic materials that are

less than 1 million to greater than 4.5 billion years old. This system is particularly useful because it has two long-lived isotopes, ^{238}U and ^{235}U ,

which decay at different rates to ^{206}Pb and ^{207}Pb , respectively, permitting the evaluation of closed-system behavior, and because both decay constants have been determined to relatively high precision (1, 2). Daughter isotope determinations from the two decay systems may also be combined to calculate a ^{207}Pb - ^{206}Pb date in concert with an assumed or measured present-day $^{238}\text{U}/^{235}\text{U}$ ratio. With recent advances in sample preparation, isotope ratio mass spectrometry, and gravimetric calibration of tracers for isotope dilution methods, the precision of an individual U-Pb or Pb-Pb age determination can exceed 0.1% (2, 3). The U-Pb chronometer has been used to improve the accuracy of other radioisotopic systems such as $^{40}\text{Ar}/^{39}\text{Ar}$ (4), Lu-Hf (5–7), Rb-Sr (8), and Re-Os (9), and to anchor extinct nuclide cosmochronometers that are used to place early solar system events in sequence. Thus, the U-Pb system has far-reaching impacts on the determination of absolute time in geological and meteoric materials.

Historically, the kinetic fractionation of U isotopes was expected to be small because of their high mass; until recently, the present-day $^{238}\text{U}/^{235}\text{U}$ ratio of all natural materials was considered invariant. In geo- and cosmochronology, a $^{238}\text{U}/^{235}\text{U}$ value equal to 137.88 has been used almost exclusively for the past 35 years (10) and is based on studies of magmatic and sedimentary uranium ore deposits (11). As published, this presumed invariant ratio and its references cannot be traced back to the International System (SI) of Units (12). More recently, IUPAC (13) recommended a value of 137.80 from the analysis of six natural ore deposits (14), confirmed by high-precision isotope ratio analyses using the IRMM-3636 ^{233}U - ^{236}U double spike (15, 16) whose isotopic composition is traceable to SI units. The invariance of the present-day $^{238}\text{U}/^{235}\text{U}$ ratio has been brought into question by studies that have demonstrated U isotopic fractionation in terrestrial materials (17–20). Such fractionation occurs during oxidation-reduction reactions (U^{VI} to/from U^{IV}), coordination change during adsorption, or leaching, and is due to thermodynamic or nuclear field shift effects (21–23). In extraterrestrial materials, excess ^{235}U may result from α decay of the short-lived ^{247}Cm (24), which has been detected in carbonaceous chondrites and their calcium- and aluminum-rich inclusions (25). Recent cosmochronology studies have highlighted the need for coupled $^{238}\text{U}/^{235}\text{U}$ and $^{207}\text{Pb}/^{206}\text{Pb}$ data sets in order to determine accurate ^{207}Pb - ^{206}Pb dates. Thus, it is crucial to re-evaluate (26) the range of natural variation of $^{238}\text{U}/^{235}\text{U}$ ratios in U-bearing minerals commonly analyzed for U-Pb age determinations.

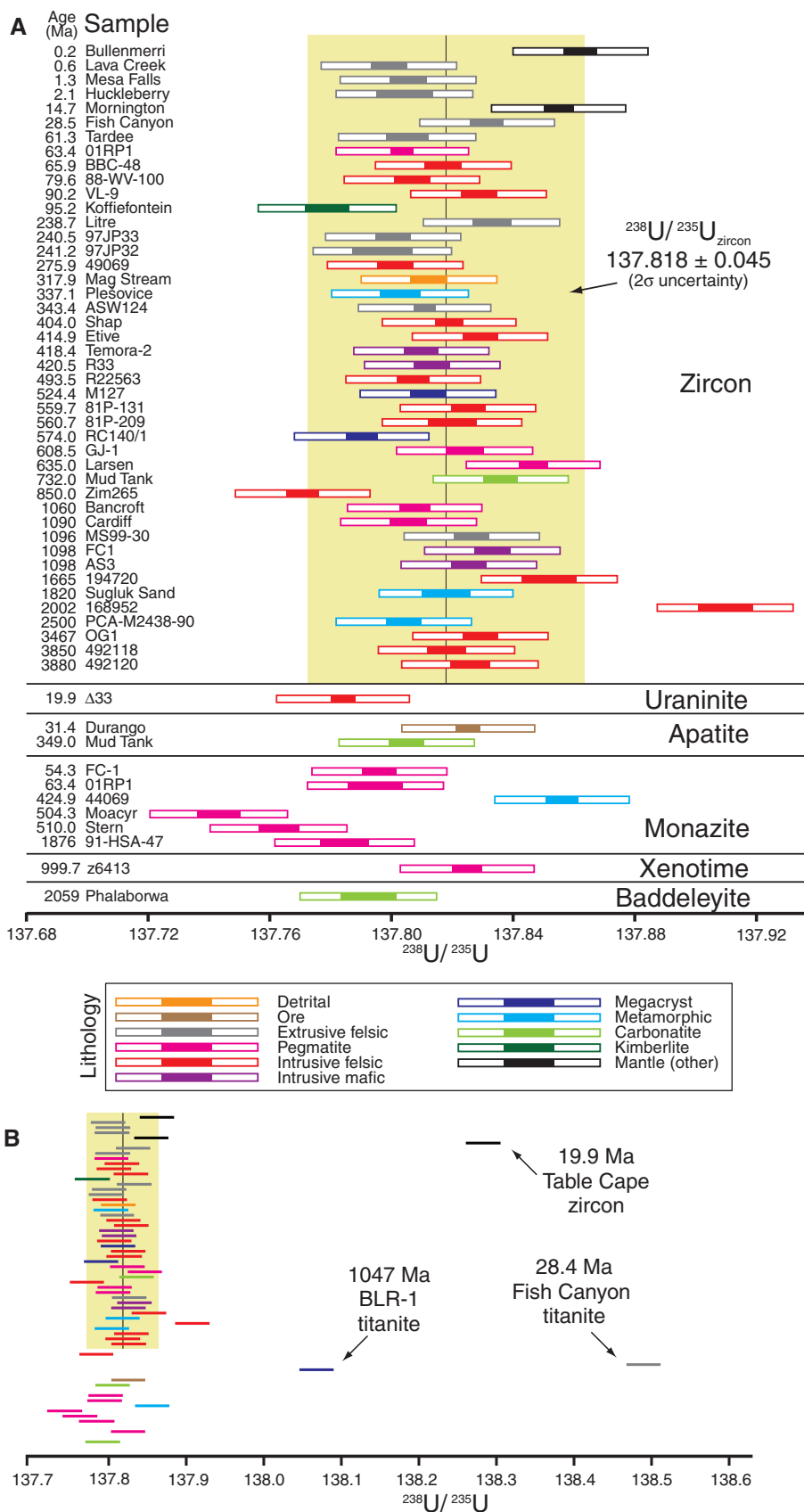


Fig. 1. (A) $^{238}\text{U}/^{235}\text{U}$ mineral summary plot including the 44 samples used to define our recommended zircon composition (represented by the yellow band). Solid and open boxes for each sample represent 2σ measured and total uncertainties, respectively. **(B)** One additional zircon sample and two additional titanite samples shown relative to the data in (A), highlighting the total range of sample compositions observed. Sample solid boxes represent 2σ total uncertainties.

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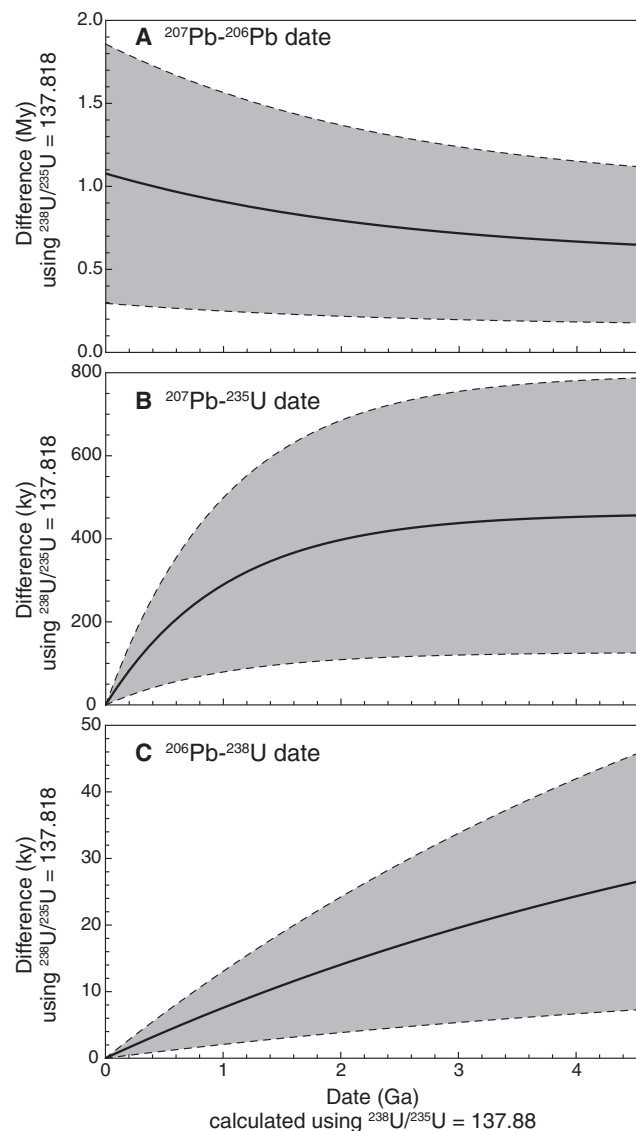
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We performed 141 $^{238}\text{U}/^{235}\text{U}$ determinations on a suite of 58 samples of U-bearing accessory minerals that are used for U-Pb geochronology (zircon, monazite, apatite, titanite, uraninite, xenotime, and baddeleyite), spanning the Quaternary to the Eoarchean and covering a diverse range of igneous and metamorphic petrogenetic settings and geographic locations (27). These data are traceable to SI units because they were measured using a gravimetrically calibrated ^{233}U - ^{236}U tracer (16); measurement uncertainties are on the order of 70 parts per million (ppm) or better.

Our data set has a >5.4 per mil (‰) range in $^{238}\text{U}/^{235}\text{U}$ (Fig. 1). The lowest measured value is 137.743 from the pegmatite-derived Moacyr monazite, and the highest is 138.490 for the Fish Canyon Tuff titanite, erupted in a large-volume silicic ash flow. Two other samples yield $^{238}\text{U}/^{235}\text{U}$ values greater than 138: BLR-1 titanite (138.068) and Table Cape zircon (138.283). The Miocene Table Cape from Tasmania may be derived from a unique, isotopically heavy reservoir more subtly expressed by Pliocene Bullenmerri (137.862) and Miocene Mornington (137.855) from Victoria, Australia, at the higher end of the main zircon $^{238}\text{U}/^{235}\text{U}$ population. These three zircon samples are likely to be mantle-derived and are sourced from regional alkaline volcanic fields. Six monazite samples have $^{238}\text{U}/^{235}\text{U}$ values from 137.743 to 137.856. Most monazite samples are sourced from pegmatites, a lithology with the potential to contain high proportions of low-temperature redox-fractionated protoliths. Resolvable $^{238}\text{U}/^{235}\text{U}$ variation between different accessory phases from the same sample—such as 01RP1 zircon and monazite (65 ppm), Mud Tank zircon and apatite (225 ppm), and Fish Canyon Tuff zircon and titanite (4.78‰)—indicates crystal-chemical and/or petrogenetic control on $^{238}\text{U}/^{235}\text{U}$ fractionation processes that operate at magmatic temperatures.

Of 45 zircon $^{238}\text{U}/^{235}\text{U}$ measurements, 44 of them are within a range of $\sim 1\%$, from 137.772 (Zim265) to 137.908 (168952). There is resolvable variation between samples, but no first-order correlation with age, petrogenetic setting, or geographic location. All five samples of uraninite, apatite, xenotime, and baddeleyite fall within the compositional range of zircon. The resolvable $^{238}\text{U}/^{235}\text{U}$ differences between samples could arise from multiple processes, including incorporation of uranium from a protolith with fractionated $^{238}\text{U}/^{235}\text{U}$ into parental magma and isotopic fractionation associated with magmatic or mineral crystallization processes. Samples of similar genetic affinity typically show agreement in $^{238}\text{U}/^{235}\text{U}$ values, suggesting isotopic homogenization within some magmatic systems (e.g., zircon from Yellowstone's Lava Creek, Mesa Falls, and Huckleberry Ridge ash-flow tuffs; Hungarian volcanic tuffs 97JP32 and 97JP33; Californian tonalites 81P-131 and 81P-209; Ontarian pegmatites Bancroft and Cardiff; Minnesotan rhyolite and anorthosites MS99-30, FC1, and AS3; Greenland tonalites 492118 and 492120; and

Fig. 2. (A to C) Plots of absolute differences between dates calculated with the consensus $^{238}\text{U}/^{235}\text{U}$ value of 137.88 and the newly defined value of 137.818 ± 0.045 for (A) zircon ^{207}Pb - ^{206}Pb dates, (B) zircon ^{207}Pb - ^{235}U dates, and (C) zircon ^{206}Pb - ^{238}U dates. Gray bands represent the 2σ uncertainty from the variability in zircon $^{238}\text{U}/^{235}\text{U}$ determined in this study. The difference is calculated by subtracting the Pb-Pb or U-Pb date calculated using $^{238}\text{U}/^{235}\text{U} = 137.818 \pm 0.045$ from the Pb-Pb or U-Pb date calculated using the $^{238}\text{U}/^{235}\text{U}$ value of 137.88. U-Pb dates are modeled using typical analytical parameters (sample/tracer $^{238}\text{U}/^{235}\text{U} = 1$) to illustrate the magnitude of differences.



monazite from British Columbian pegmatites FC-1 and 01RP1).

Of the zircon samples measured, 44 of 45 define an approximately normally distributed population with a mean of 137.818 and standard deviation of 0.022, with population parameters calculated using (28), which corrects for the expected additional dispersion from analytical uncertainties. We propose that this average zircon value and its associated variability ($137.818 \pm 0.045/0.050$, 2σ), which is traceable to the SI system of units, is applicable for the majority of U-Pb determinations and, in the absence of an independently determined $^{238}\text{U}/^{235}\text{U}$ value, should be adopted for future use in U-Pb geochronology of zircon. The first uncertainty reported reflects the variability found in nature; the second additionally incorporates systematic uncertainties in the isotopic composition of the tracer. Other phases, such as monazite and titanite, require further assessment of their $^{238}\text{U}/^{235}\text{U}$ variability.

Adoption of the average $^{238}\text{U}/^{235}\text{U}_{\text{zircon}}$ value of 137.818 ± 0.045 for use in zircon geochro-

nology will decrease ^{207}Pb - ^{206}Pb , ^{207}Pb - ^{235}U , and ^{206}Pb - ^{238}U dates relative to those calculated using the conventional $^{238}\text{U}/^{235}\text{U}$ value of 137.88 (12, 18). For ^{207}Pb - ^{206}Pb dates, the $^{238}\text{U}/^{235}\text{U}$ ratio is implicit in the age equation and the magnitude of the difference is largest, changing gradually from ~ 1 million years for samples dated 100 million years ago (Ma) to $\sim 700,000$ years for samples dated 4 billion years ago (Ga) (Fig. 2A). The observed variability in $^{238}\text{U}/^{235}\text{U}_{\text{zircon}}$ may limit precision for >1 Ga zircon samples with no independent $^{238}\text{U}/^{235}\text{U}$ constraint. For ^{207}Pb - ^{235}U and ^{206}Pb - ^{238}U dates (Fig. 2, B and C), the mineral $^{238}\text{U}/^{235}\text{U}$ is used in tracer subtraction and fractionation correction calculations for tracers enriched in ^{235}U that are commonly used in high-precision U-Pb geochronology (supplementary text). For typical sample/tracer $^{238}\text{U}/^{235}\text{U}$ ratios close to unity, the biases are $<500,000$ years for ^{207}Pb - ^{235}U dates and $<30,000$ years for ^{206}Pb - ^{238}U dates younger than 4.4 Ga. For Phanerozoic zircons, the change in ^{206}Pb - ^{238}U dates is <4000 years.

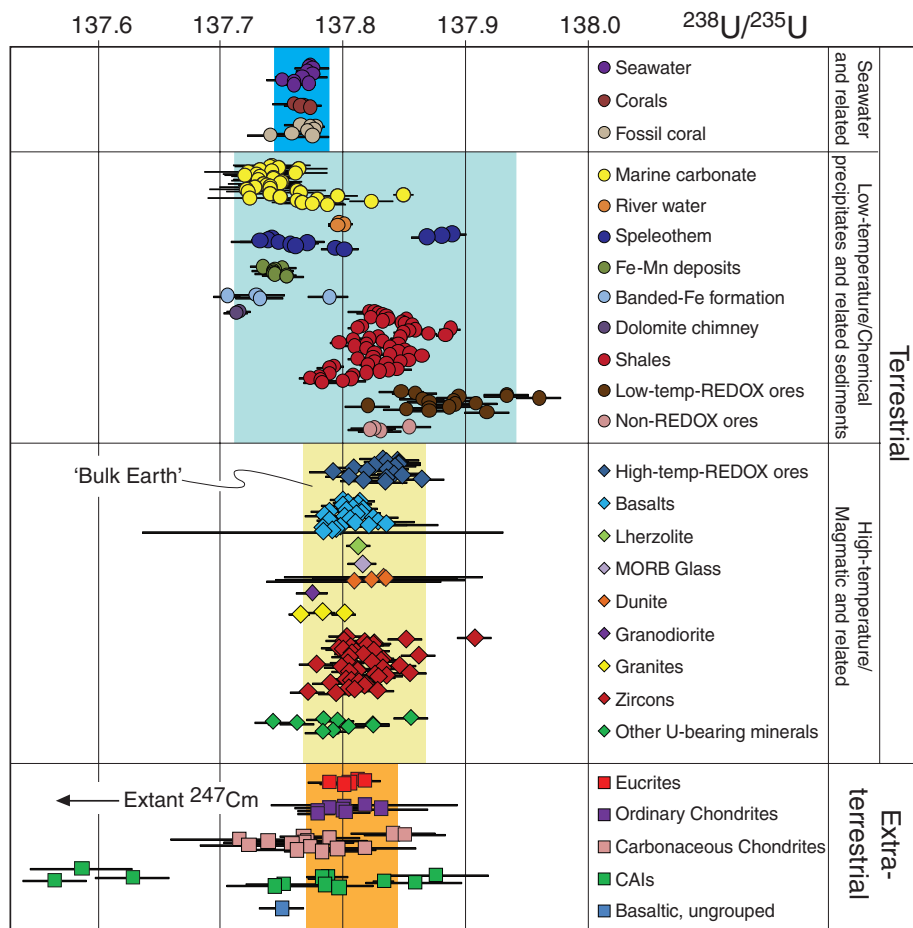


Fig. 3. Compilation of $^{238}\text{U}/^{235}\text{U}$ data obtained on a wide variety of geological and extraterrestrial materials. The “bulk Earth” field (yellow band) is based on the terrestrial/high-temperature data set, consistent with eucrites and ordinary chondrites. Seawater and related precipitates show a systematic enrichment in ^{235}U relative to the “bulk Earth” field. Data are from literature sources (17, 18, 25, 30–33, 39–41) and this study (zircon and other U-bearing minerals).

High-precision U-Pb isotope analyses of closed-system zircon and xenotime have also been exploited to derive a more precise ^{235}U decay constant ($\lambda^{235}\text{U}$) value (2, 3, 29). In this approach, the systematic bias between ^{206}Pb - ^{238}U dates and ^{207}Pb - ^{235}U dates is minimized by solving for a new value of $\lambda^{235}\text{U}$ relative to the more precisely determined $\lambda^{238}\text{U}$ (1). These studies have used an assumed $^{238}\text{U}/^{235}\text{U}$ value of 137.88 (2, 3, 29). Mattinson (2) discussed the effects of intermediate daughter disequilibrium, mass isotopic fractionation, tracer calibrations, and $^{238}\text{U}/^{235}\text{U}$ on the accuracy and precision of U-Pb analyses. Our data set includes a subset of samples dated in these previous studies and allows us to better evaluate the impact of a more accurate $^{238}\text{U}/^{235}\text{U}$ on uranium decay constant intercalibration. Accepting the published U-Pb data (2, 3) and the samples’ unique $^{238}\text{U}/^{235}\text{U}_{\text{zircon}}$ values determined in this study yields a recalculated $\lambda^{235}\text{U} = 0.98531$ per billion years (fig. S10), intermediate between the Jaffey *et al.* (1) counting experiment value and the closed-system U-Pb reevaluations using $^{238}\text{U}/^{235}\text{U} = 137.88$ (2, 3). However, a robust $\lambda^{235}\text{U}$ can only be

determined with U-Pb analyses using a tracer calibration that is traceable to SI units and free of other potential sources of bias, so we refrain from suggesting that this value be adopted at present and urge caution in abandoning the Jaffey *et al.* (1) $\lambda^{235}\text{U}$ determination until such a data set has been generated and evaluated.

An emerging $^{238}\text{U}/^{235}\text{U}$ data set for a wide range of rocks, minerals, and meteorites is now available (17, 18, 25, 30–33) (Fig. 3). Given that natural $^{238}\text{U}/^{235}\text{U}$ variation has been demonstrated up to $\sim 0.13\%$ (17, 18), it might be expected that a corresponding variation be observed in the U-bearing mineral data set, because $^{238}\text{U}/^{235}\text{U}$ fractionated material from low-temperature environments is incorporated into higher-temperature systems through crustal recycling processes. A first-order observation from the compiled $^{238}\text{U}/^{235}\text{U}$ data is that materials formed in near-surface environments (e.g., chemical precipitates) record a wider range than crustal rocks and minerals formed in higher-temperature magmatic environments (17, 18). This suggests that uranium in magmatic and derived crustal reservoirs (e.g., siliciclastic sediments) is isotopically well mixed relative to

uranium in materials formed in near-surface environments, and that the low-temperature materials with highly fractionated $^{238}\text{U}/^{235}\text{U}$ constitute volumetrically minor reservoirs that are continually and efficiently homogenized via crustal recycling processes. Second, modern seawater and Quaternary seawater precipitates are systematically lower than the “bulk Earth” $^{238}\text{U}/^{235}\text{U}$ composition, indicating ^{235}U enrichment in the marine reservoir. Seawater enrichment in ^{234}U (34) by $\sim 147\%$ relative to radioactive secular equilibrium is a well-known consequence of radioactive α -recoil processes and the preferential release of the non-lattice-bound ^{234}U daughter nuclide into the hydrological environment (35, 36). Previous studies demonstrated a broad positive correlation between ^{234}U and ^{235}U depletion in near-surface environments (17), but an α -recoil-related mechanism cannot account for $^{235}\text{U}/^{238}\text{U}$ fractionation, as both are lattice-bound. Zircon acid leaching experiments carried out in this study also recorded a systematic enrichment in ^{235}U in the leachate (27), which suggests preferential leaching of lattice-bound ^{235}U , and similar fractionation has been detected in euxenite leaching experiments (17). By analogy, we suggest leaching of lattice-bound ^{235}U during long-term chemical weathering of exposed crustal rocks as a viable mechanism to explain ^{235}U enrichment in seawater.

Uranium-bearing accessory minerals from a wide range of crustal and mantle-derived rock types record a restricted (0.07%) range of $^{238}\text{U}/^{235}\text{U}$ values that encompasses nearly all published $^{238}\text{U}/^{235}\text{U}$ values determined on high-temperature (i.e., magmatic) rocks and minerals including granites, dunite, and basalts (Fig. 3). The overlap in $^{238}\text{U}/^{235}\text{U}$ values for crust and upper mantle-derived lithologies indicates no resolvable fractionation between the terrestrial reservoirs sampled. Furthermore, despite $^{238}\text{U}/^{235}\text{U}$ values of meteoritic material recording excess ^{235}U derived from extant ^{247}Cm (24, 25, 37), ordinary chondrites, eucrites, and the upper limits for calcium/aluminum inclusions and carbonaceous chondrites overlap with the field delineated by terrestrial crust and mantle materials (30, 32, 33). This agreement suggests that a uniform $^{238}\text{U}/^{235}\text{U}$ was achieved relatively early during planetary accretion and that U isotope compositions in the high-temperature terrestrial crust and upper mantle are also likely to apply to the lower mantle, thereby defining the bulk silicate Earth isotope composition. In light of the agreement of terrestrial and meteoritic isotope compositions and current Earth accretion models (38), this average $^{238}\text{U}/^{235}\text{U}$ value of 137.818 ± 0.050 would also represent the isotope composition of “bulk Earth.”

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Supporting Online Material

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A Shorter ^{146}Sm Half-Life Measured and Implications for ^{146}Sm - ^{142}Nd Chronology in the Solar System

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The extinct p -process nuclide ^{146}Sm serves as an astrophysical and geochemical chronometer through measurements of isotopic anomalies of its α -decay daughter ^{142}Nd . Based on analyses of $^{146}\text{Sm}/^{147}\text{Sm}$ α -activity and atom ratios, we determined the half-life of ^{146}Sm to be 68 ± 7 (1 σ) million years, which is shorter than the currently used value of 103 ± 5 million years. This half-life value implies a higher initial ^{146}Sm abundance in the early solar system, $(^{146}\text{Sm}/^{144}\text{Sm})_0 = 0.0094 \pm 0.0005$ (2 σ), than previously estimated. Terrestrial, lunar, and martian planetary silicate mantle differentiation events dated with ^{146}Sm - ^{142}Nd converge to a shorter time span and in general to earlier times, due to the combined effect of the new ^{146}Sm half-life and $(^{146}\text{Sm}/^{144}\text{Sm})_0$ values.

The α decay of ^{146}Sm serves as a clock for determining the chronology of solar system formation (1) and planetary differentiation (2) and can reveal insights into p -process nucleosynthesis of solar ^{146}Sm (3). The correlation between anomalies in isotopic abundances of ^{142}Nd and the Sm content, first observed (4) in meteorites, provided evidence for extinct

^{146}Sm (entirely decayed to ^{142}Nd). ^{142}Nd anomalies, primarily positive relative to the chondritic uniform reservoir (CHUR), were observed in meteorite parent bodies (5–7), Earth (8–11), the Moon (12–14), and Mars (15–18). They are attributed to a small fractionation during partial melting or solidification favoring Sm in the solid phase in the silicate mantle owing to the slightly higher incompatibility of Nd, while ^{146}Sm was still live (not entirely decayed). Caro et al. (18) inferred in addition that the Sm/Nd ratio of the bulk Earth, Moon, and Mars is 5 to 10% higher than that in the CHUR, which accounts for part of the positive anomalies, possibly due to fractionation and collisional erosion (2, 14, 18, 19).

The half-life of ^{146}Sm , which sets the scale of the ^{146}Sm - ^{142}Nd clock, was previously measured four times (20–23) between 1953 and 1987, and its currently adopted value, derived from the

works of Friedman et al. (22) and Meissner et al. (23), is 103 ± 5 million years (My). Considering the range of measured ^{146}Sm half-life ($t_{1/2}^{146}$) values [–50 My (20), 74 ± 15 My (21), and 103 ± 5 My (22, 23)] and its importance in solar system chronology (2), we redetermined $t_{1/2}^{146}$ by measuring the α -activity ratio and atom ratio of ^{146}Sm to those of naturally occurring ^{147}Sm in activated samples of ^{147}Sm and using the equation $t_{1/2}^{146} = \frac{A_{147}}{A_{146}} \times \frac{N_{146}}{N_{147}} \times t_{1/2}^{147}$. Here, $t_{1/2}^{147}$ denotes the ^{147}Sm α -decay half-life, 107.0 ± 0.9 billion years (Gy) (24), and A_A and N_A denote the α activity and atom number of ^ASm in the sample, respectively. The ratio measurement eliminates most systematic uncertainties in determining the α activity due to detector efficiency and geometrical acceptance. We produced three independent ^{146}Sm source materials by activating isotopically enriched ^{147}Sm targets via the following nuclear reactions: (i) $^{147}\text{Sm}(\gamma, n)^{146}\text{Sm}$ (using 50-MeV electron bremsstrahlung radiation); (ii) $^{147}\text{Sm}(p, 2n)^{146}\text{Sm}$ reaction; and (iii) fast-neutron activation $^{147}\text{Sm}(n, 2n)^{146}\text{Sm}$ (25). We prepared spectroscopic α sources (20 to 100 μg) from the three activations and counted them over several months using a silicon surface-barrier detector at Kanazawa University (Fig. 1) (25), thus determining the α -activity ratio. For the determination of the $^{146}\text{Sm}/^{147}\text{Sm}$ atom ratio, we used accelerator mass spectrometry (AMS) at the ATLAS facility (Argonne National Laboratory) because of the need to discriminate isobaric ^{146}Nd interferences, observed to be critical in our experiment when using thermal ionization mass spectrometry (26). The AMS isobaric separation was accomplished by the combined action of the high energy of the Sm ions after acceleration and the use of a gas-filled magnetic spectrograph (27, 28) (Fig. 1). Two methods were used to determine the $^{146}\text{Sm}/^{147}\text{Sm}$

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atom ratio: the ^{146}Sm counting rate versus either the $^{147}\text{Sm}^{22+}$ charge current or the quantitatively attenuated ^{147}Sm counting rate, giving consistent results [see (25, 29) for details of sample preparation and methods of measurement].

Our measured ^{146}Sm half-life is shorter than the adopted value by a factor of 0.66 ± 0.07 (1σ) (unweighted average and standard deviation) (Fig. 2). This corresponds to a ^{146}Sm half-life of 68 ± 7 My (1σ); the systematic error (1%) as-

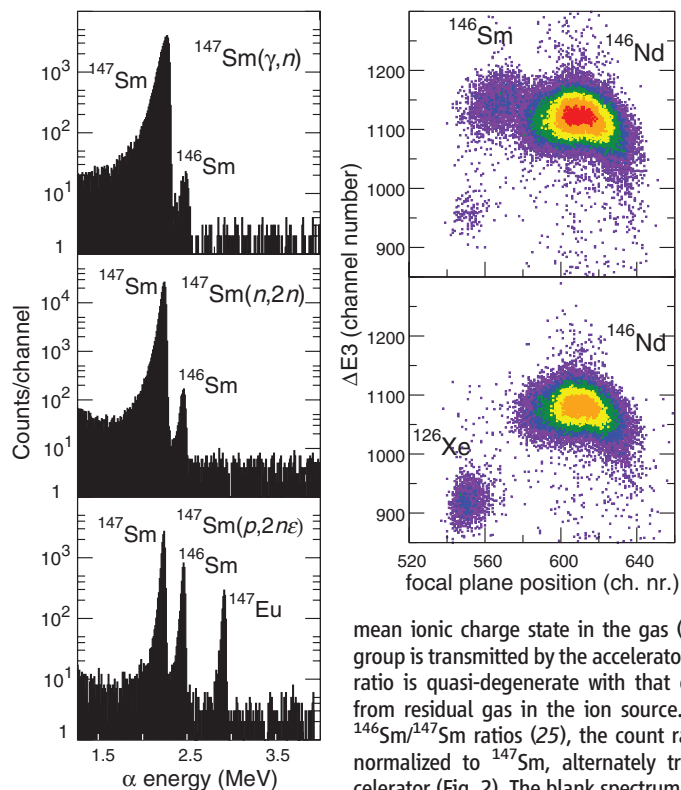
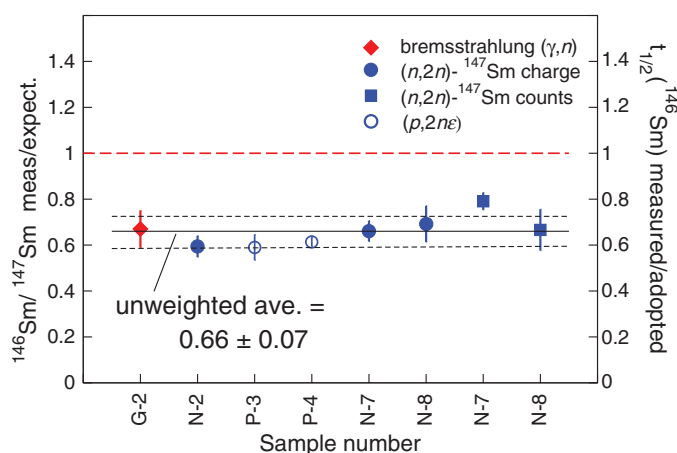


Fig. 1. (Left) α spectra from ^{147}Sm activated via (γ, n) , $(n, 2n)$ and $(p, 2ne)$ reactions, determining the $^{146}\text{Sm}/^{147}\text{Sm}$ activity ratio; (right) identification spectra measured for a $(n, 2n)$ -activated sample (top) and nonactivated ^{147}Sm (bottom). The spectra display energy loss versus position signals from a detector along the focal plane of a gas-filled magnetic spectrograph. The device separates in position (magnetic rigidity) ^{146}Sm ions from ^{146}Nd isobaric ions (originating from chemical impurities in the sample or in ion source structural material), owing to their different

mean ionic charge state in the gas (25). The observed $^{126}\text{Xe}^{19+}$ group is transmitted by the accelerator because its charge-to-mass ratio is quasi-degenerate with that of $^{146}\text{Sm}^{22+}$ and originates from residual gas in the ion source. In order to determine the $^{146}\text{Sm}/^{147}\text{Sm}$ ratios (25), the count rate in the ^{146}Sm group was normalized to ^{147}Sm , alternately transported through the accelerator (Fig. 2). The blank spectrum shown for the ^{147}Sm sample (lower right) corresponds to a ratio $^{146}\text{Sm}/^{147}\text{Sm} < 10^{-11}$.

Fig. 2. Double ratios of the $^{146}\text{Sm}/^{147}\text{Sm}$ atom ratio measured by AMS to that expected in the same sample from its $^{146}\text{Sm}/^{147}\text{Sm}$ α -activity ratio, using the currently adopted ^{146}Sm half-life, 103 My (22, 23). The double ratio is equivalent to the ratio of our measured ^{146}Sm half-life to the currently adopted value (right vertical axis). The dashed red line corresponds to a ratio of 1. The notations G-x, N-x, P-x on the horizontal axis



denote various independently prepared samples from the bremsstrahlung, neutron, and proton irradiations, respectively. The plotted value for each sample is the unweighted mean and standard deviation of the double ratios measured in repeat measurements of that sample (see table S1 and figs. S4 and S5 for individual values). The error bars represent random statistical errors in AMS ion counting and α activity, uncertainty in dilution factors, and random errors in ion transmission. The unweighted mean and standard deviation of the double ratios shown, 0.66 ± 0.07 , correspond to a ^{146}Sm half-life of 68 ± 7 My. The diamond and circles represent values measured by normalizing ^{146}Sm counts to $^{147}\text{Sm}^{22+}$ charge, and squares represent values obtained by normalizing ^{146}Sm counts to a quantitatively attenuated ^{147}Sm beam measured in the same detector as ^{146}Sm ions, avoiding possible systematic uncertainties in the charge current measurement or in transmission efficiency between the Faraday cup and detector (25, 29).

sociated with the ^{147}Sm half-life (24) is small as compared to the random errors. Our value is shorter by $\sim 30\%$ than the values (102.6 ± 4.8 My and 103.1 ± 4.5 My) from (22, 23), respectively, and is consistent (within larger uncertainties) with the two earlier works of Dunlavey and Seaborg (20) and Nurmia *et al.* (21) (~ 50 My and 74 ± 15 My, respectively). Different from these previous measurements, our AMS determination of ^{146}Sm is free of isobaric contributions (^{146}Nd) which can cause contamination in rare-earth chemistry and mass spectrometry. The determination of an isotopic ratio ($^{146}\text{Sm}/^{147}\text{Sm}$) makes our experiment also insensitive to systematic effects inherent in measuring an absolute number of ^{146}Sm atoms, as was done in previous measurements.

Our ^{146}Sm half-life value implies a reevaluation of the initial $(^{146}\text{Sm}/^{144}\text{Sm})_0$ ratio in the solar system and reinterpretation of the ^{146}Sm source and dating of planetary differentiation events (Table 1). Although evidence for extinct ^{146}Sm has been found in many meteorites, the majority show evidence of chemical and isotopic re-equilibration, which potentially altered the initial ratio (5–7). In a recent study, Boyet *et al.* (30) selected a number of meteorites that appear to have remained closed Sm-Nd systems. Using the individual $^{146}\text{Sm}/^{144}\text{Sm}$ ratios of these meteorites and ^{147}Sm - ^{143}Nd ages, they redetermined the initial solar system value $(^{146}\text{Sm}/^{144}\text{Sm})_0 = 0.0085 \pm 0.0007$ (2σ) [compared to 0.008 ± 0.001 (2σ) used in most studies (6)]. As a basis for comparison, we fitted the same set of data using our measured ^{146}Sm half-life and derived an initial ratio $(^{146}\text{Sm}/^{144}\text{Sm})_0 = 0.0094 \pm 0.0005$ (2σ) (Fig. 3). A fit taking into account uncertainties in both age and isotopic ratio leads, however, to the less precise value $0.0094^{+0.0018}_{-0.0015}$ (2σ). The determination of an experimental ^{146}Sm decay curve from meteoritic data would require precision in determining ^{146}Sm abundances that is beyond present capability or more data on meteorites dated to < 4500 million years ago (Ma). The initial $(^{146}\text{Sm}/^{144}\text{Sm})_0$ ratio results from the decay of ^{146}Sm in the interstellar medium (ISM) during an isolation interval Δ , and both of these quantities depend on the ^{146}Sm half-life. The initial ratio can be expressed (*I*, *3I*) in terms of the ISM abundance $(^{146}\text{Sm}/^{144}\text{Sm})_{\text{ISM}}$ as

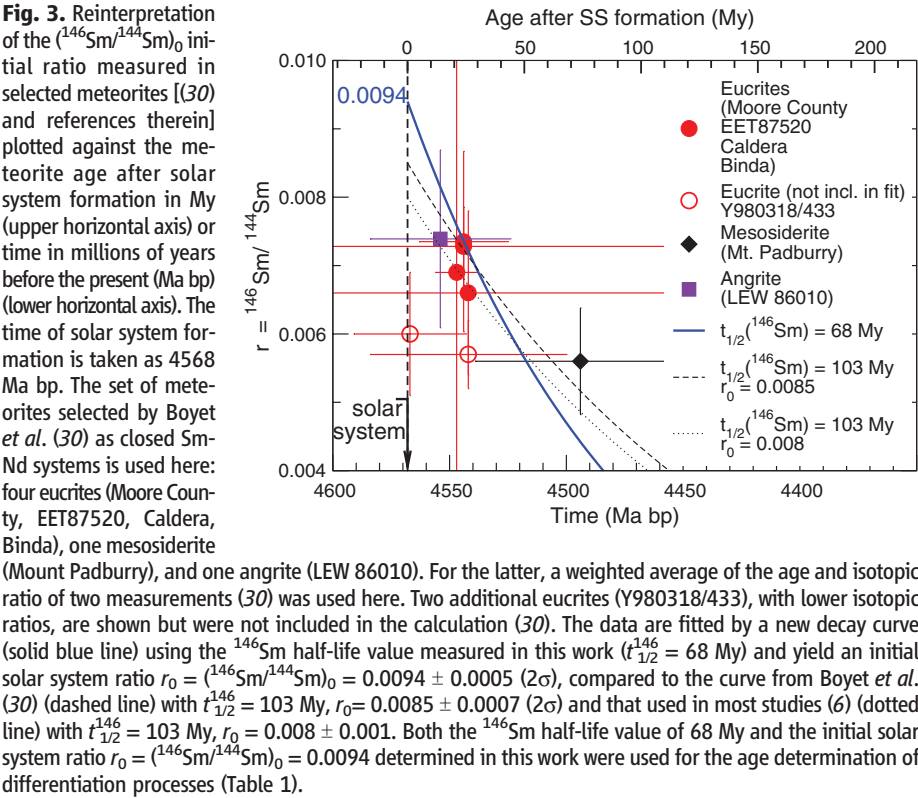
$$\left(\frac{^{146}\text{Sm}}{^{144}\text{Sm}}\right)_0 = \left(\frac{^{146}\text{Sm}}{^{144}\text{Sm}}\right)_{\text{ISM}} \exp(-\Delta/\tau) = \frac{P^{146}}{P^{144}} \kappa \frac{\tau}{T} \exp(-\Delta/\tau) \quad (1)$$

where P^{146}/P^{144} denotes the $^{146}\text{Sm}/^{144}\text{Sm}$ p -process production ratio (taken as ~ 1), $\tau = 98$ My is the ^{146}Sm mean life determined in this work, $T \sim 10$ Gy is the presolar age of the Galaxy, and $\kappa = p(T)/\langle p \rangle$ is the ratio of p -process rate just before solar system formation to the average p -process rate [κ ranging from 1 for the uniform-production (closed box) model (*I*) to ~ 2.7 for an open-box model (*3I*) with Galactic-disk enrichment in

Table 1. The effect of the shorter ^{146}Sm half-life on the estimated time of differentiation events.

Planetary body	Sample/Mantle differentiation event	Reference	Time after start of solar system formation (My)	
			From ref. in column 3*	Revised in present work†
Earth	Terrestrial rocks 18 ppm higher than CHUR / Depleted–enriched reservoirs	(9)	≤30	No change
	Archean array, Isua, Greenland / Depleted source	(2)	170‡	120§
	Nuvvuagittuq greenstone belt, northern Quebec, Canada / Enriched source	(11)	287 ⁺⁸¹ _{–53}	205 ⁺⁵⁴ _{–35} ¶
Moon	Lunar array / LMO solidification#	(2)	242 ± 22	170 ± 15**
	FAN 60025 / LMO solidification#††	(31)	250 ⁺³⁸ _{–30}	175 ⁺²⁵ _{–20} ‡‡
Mars	Nakhlites / Solidification of depleted source	(16)	8 – 25§§	Favors young age
	Enriched shergottites / Solidification of source	(17)	~110¶¶	~90##

*Derived in the original studies (column 3) using $t_{1/2}^{146} = 103$ My (22, 23), $(^{146}\text{Sm}/^{144}\text{Sm})_0 = 0.008$ (6), $t_0 = 4567$ Ma. †Derived using the value presented in this work. ‡Using $(^{146}\text{Sm}/^{144}\text{Sm})_0 = 0.0085$. §Reinterpretation of fig. 3 of Caro (2). ||Measured in faux amphibolite and gabbro (>3800 Ma before the present). ¶Reinterpretation of fig. 3 of O’Neil *et al.* (11). #LMO = lunar magma ocean. **Reinterpretation of fig. 8 of Caro (2). ††FAN = ferroan anorthosite. ‡‡Reinterpretation of fig. 2b of Borg *et al.* (32). §§Calculated a depleted source of $^{147}\text{Sm}/^{144}\text{Nd} \sim 0.255$ to 0.266 ($^{180}\text{Hf}/^{183}\text{W} \sim 22$ to 43) for the martian mantle source. ||Calculated a less depleted source of $^{147}\text{Sm}/^{144}\text{Nd} \sim 0.245$ ($^{180}\text{Hf}/^{183}\text{W} \sim 20$) for the martian mantle source (fig. S5) (25). The young age may be in line with the recent finding that Mars accreted within ~ 4 My (33). ¶¶Calculated assuming that the data form a mixing line and the source has a CHUR Sm/Nd ratio. See fig. S5 (25). Alternative interpretation of the data line as an isochron and a source with a superchondritic Sm/Nd ratio gives an age of ~40 My (18). ##See fig. S6 (25).



low-metallicity gas]. The isolation interval Δ calculated from Eq. 1 with our half-life and $(^{146}\text{Sm}/^{144}\text{Sm})_0$ values is reduced by factor of ~ 2.5 to 20 from previous estimates to ~5 My in the closed-box model and ~100 My in the open-box model.

The ^{146}Sm decay curve using $t_{1/2}^{146} = 68$ My, $(^{146}\text{Sm}/^{144}\text{Sm})_0 = 0.0094$ and the two other decay curves with $t_{1/2}^{146} = 103$ My [with $(^{146}\text{Sm}/^{144}\text{Sm})_0 = 0.0085$ (30) and 0.008 (6)] intersect in the range of 30 to 50 My (Fig. 3). This reduces the age of late events (>50 My) (age is measured in this

work relative to the birth of the solar system) but has a minor effect on earlier events (Table 1). ^{146}Sm observations in terrestrial samples can be divided into two groups: (i) Most terrestrial rocks display a $^{142}\text{Nd}/^{144}\text{Nd}$ ratio higher by ~18 parts per million (ppm) than in the CHUR (9), and (ii) anomalies in the $^{142}\text{Nd}/^{144}\text{Nd}$ ratio relative to the terrestrial standard, both positive, in rocks from Greenland and Australia (2, 10), and negative, in rocks from northern Quebec (2, 11). Two explanations were offered for the (i) anomalies: that the mantle had to differentiate within ≤30 My (2, 9) or that Earth (and the Moon and Mars) have a bulk Sm/Nd ratio 5 to 10% higher than that of the CHUR (2, 14, 18), and neither explanation is affected by the new values presented here. At the same time, ages derived from (ii) change significantly. One such example is the differentiation age of the depleted mantle source of Archean rocks from Isua (Greenland) (2): The interpretation of this array as an isochron (2) leads now to an age of 120 My rather than 170 My. The revised age improves the agreement with the 70- to 170-My ^{176}Lu – ^{176}Hf differentiation age derived from the Hadean Jack Hills zircons (Australia) (2), strongly suggesting that one global differentiation event is sampled in both cases. Another example is the isochron that dates the differentiation of the enriched mantle source of rocks from Quebec (11), whose estimated age decreases from 287^{+81}_{-53} to 205^{+54}_{-35} My. An array of lunar rocks (12, 13) has been similarly interpreted as an isochron (2), which dates the solidification of the lunar magma ocean. The isochron-inferred age decreases with the new values from 242 ± 22 to 170 ± 15 My. A recent study by Borg *et al.* (32) dated a lunar sample, ferroan anorthosite (FAN) 60025, by three methods: Pb–Pb, ^{147}Sm – ^{143}Nd , and ^{146}Sm – ^{142}Nd . The Pb–Pb and ^{147}Sm – ^{143}Nd systems gave consistent ages of 208.8 ± 2.4 and 201 ± 11 My, respectively, but the ^{146}Sm – ^{142}Nd age, 250^{+38}_{-30} My, showed a discrepancy. Our value of ^{146}Sm half-life revises the latter to 175^{+25}_{-20} My (Table 1) and removes most of this discrepancy, bringing the ^{146}Sm – ^{142}Nd age into agreement with the ^{147}Sm – ^{143}Nd age. This is an important point considering that FAN 60025 is now the only rock dated precisely enough with the different systems to trace their decay. The new ^{146}Sm age of FAN60025 remains in accord with the revised younger age of the lunar array isochron.

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Control of Sleep by Cyclin A and Its Regulator

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How and why the brain reversibly switches from a waking to a sleep state remain among the most intriguing questions in biology. We show that cyclin A (CycA) and regulator of cyclin A1, essential cell cycle factors, function in postmitotic neurons to promote sleep in *Drosophila melanogaster*. Reducing the abundance of CycA in neurons delayed the wake-sleep transition, caused multiple arousals from sleep, and reduced the homeostatic response to sleep deprivation. CycA is expressed in ~40 to 50 neurons in the adult brain, most of which are intermingled with circadian clock neurons, suggesting functional interactions among neurons controlling sleep and circadian behavior.

Prolonged intervals of rest in *Drosophila* share many features with mammalian sleep (1, 2). Flies spend a large portion of their waking time moving, and the locomotor assay developed for circadian behavior (3) can therefore be used to screen for sleep mutants in this animal. A bout of inactivity lasting 5 min or longer is associated with an increased arousal threshold (1, 2), as well as changes in neural activity (4), and is used to define sleep in the fly. Since the discovery of sleep in *Drosophila* a decade ago (1, 2), the results of several genetic screens for sleep factors have been reported, and many of the genes identified have conserved roles across species [reviewed in (5)]. A surprisingly large fraction of these genes are ion channels or their regulators, or are involved with broadly acting neurotransmitter pathways. These findings led to the hypothesis that there are no dedicated sleep genes but rather that sleep is regulated by genes that control normal neuronal function [reviewed in (5)].

We decided to screen for sleep genes solely within the nervous system, bypassing the potential confounding effects of these genes in other tissues. We screened ~4000 UAS-RNA interference (UAS-RNAi) lines using the pan-neuronal driver *elavGal4* to knock down gene expression, while simultaneously driving the expression of *UAS-Dicer2* to enhance RNAi efficacy (6). After three retesting rounds, we identified ~20 genes whose RNAi-mediated neuronal depletion led to reproducible changes in sleep pattern, quantity, or both. One sleep-promoting factor we isolated was *Rca1* (regulator of cyclin A1), a conserved, essential cell cycle gene (7–10).

Depletion of *Rca1* from neurons (*elavGal4* + *UAS-Rca1-RNAi*, denoted “*elav>Rca1-RNAi*”) decreased the amount of total daily sleep (Figs. 1A and 2F), without affecting the levels of activity per waking minute (Fig. 1B). It did so by decreasing the duration of individual sleep episodes (sleep bouts, Fig. 1C). The number of sleep episodes was slightly increased in *elav>Rca1-RNAi* animals (Fig. 1D), likely because of the highly fragmented nature of their sleep. The net reduction in sleep duration understates the severity of the *elav>Rca1-RNAi* phenotype. For example, *elav>Rca1-RNAi* flies that showed no net

change in daily sleep often failed to display behavior that is organized into prolonged intervals of rest and activity seen in the control flies (see examples of locomotor traces in Fig. 1A).

Drosophila Rca1 is homologous to the mammalian early mitotic inhibitor (Emi1) (9, 10) and plays a critical role in preventing premature cyclin degradation (8). Cyclins regulate cell cycle progression through activation of specific cyclin-dependent kinases (CDKs). The main target of *Drosophila* Rca1 regulation is cyclin A (CycA) (7), so we tested whether depletion of CycA might affect sleep. Indeed, expression of *CycA-RNAi* in neurons (*elavGal4* + *UAS-CycA-RNAi*, denoted “*elav>CycA-RNAi*”) caused a decrease in the time animals spent sleeping (Fig. 2, A and F) without significantly affecting levels of activity per minute while awake (Fig. 2B). This reduction was a consequence of decreased sleep bout duration (Fig. 2C), whereas the number of sleep episodes was slightly increased (Fig. 2D).

We noticed that the ectopic overexpression of Rca1 or CycA in neurons produced a small and rough eye phenotype. A similar eye phenotype is associated with a mutation in a negative regulator of *CycA* and was used in a modifier screen to identify new genes involved in cell cycle regulation (7). We used this morphological readout of CycA abundance to confirm that the *UAS-Rca1-RNAi* and *UAS-CycA-RNAi* lines were effective, by showing that they suppressed eye phenotypes associated with Rca1 or CycA overexpression, respectively (fig. S1, A and B). Furthermore, *UAS-Rca1-RNAi* also suppressed the eye phenotypes resulting from CycA overexpression (fig. S1C), indicating that Rca1 can stabilize CycA protein in neurons.

Flies homozygous for *CycA* null alleles (*CycA*[−]) do not survive and could not be examined. However, *CycA*[−] heterozygotes are viable and showed a decrease in total sleep amounts, a phenotype further enhanced by expression of *CycA-RNAi* in this background (Fig. 2E), even in the absence of

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Dicer2 overexpression. We observed an additive effect between the heterozygous *CycA*⁺ mutation and neuronal *Rca1-RNAi* as well (Fig. 2E). The reduction in total sleep seen in these experiments may not fully reflect the influence of *CycA* and *Rca1* in sleep control, as *CycA* protein was still detectable in the brains of all of our experimental genotypes.

As *CycA* is thought to be the main target of *Rca1* regulation during the cell cycle, and our behavioral and genetic analyses indicated a similar relation with respect to the effects of these genes on sleep, we focused our subsequent studies on *CycA*.

It took significantly longer for *elav>CycA-RNAi* flies to fall asleep after lights went off than it did for the controls (Fig. 3A). This was not the case for all genes that were identified in our screen. This increased latency to sleep suggests that a mechanism regulating wake-sleep transition is disrupted in *elav>CycA-RNAi* flies. To determine if sleep can be homeostatically controlled in these animals, we examined their response to sleep deprivation. We mechanically stimulated the flies during the night and examined their sleep pattern during the subsequent morning, when “rebound” sleep is normally seen in wild-type flies that have been sleep-deprived the night before (1, 2). Although we saw some increase in rebound sleep in flies of all the genotypes tested, this response was reduced in *elav>CycA-RNAi* flies (Fig. 3, B and C), suggesting a defective sleep homeostat. In accordance with the idea

that sleep serves an essential function, neuronal depletion of *CycA* shortens life span (Fig. 3D), presumably due to chronic sleep deprivation experienced by these animals. However, it is also possible that *CycA* contributes to life span through other functions in postmitotic neurons.

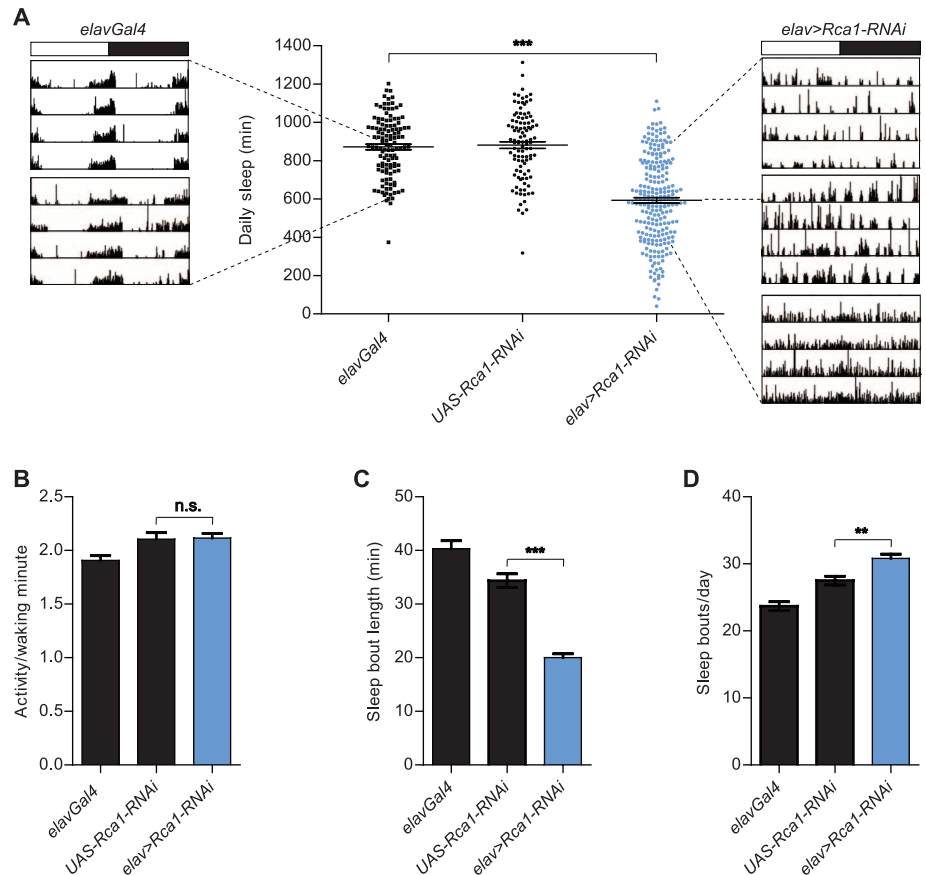
Sleep is regulated in a circadian fashion, so we wondered whether some of the sleep defects we observed in *elav>CycA-RNAi* animals resulted from a disrupted circadian clock. Most *elav>CycA-RNAi* flies showed robust rhythms in constant darkness, with normal periods (figs. S2A and S3A). Circadian rhythmicity was observed even in animals with severe sleep defects (fig. S2, B to D). Furthermore, *elav>CycA-RNAi* flies eclosed with strong circadian rhythms (fig. S3B). Finally, Period (PER), a core component of the circadian clock (11), accumulated rhythmically in the pacemaker neurons of *elav>CycA-RNAi* animals (fig. S3C). Thus, the sleep defects observed in *elav>CycA-RNAi* animals appear not to reflect circadian clock anomalies.

Staining with two different antibodies to *CycA* showed a restricted expression pattern in adult brains (fig. S4). Both antibodies detected the same ~12 dorsal neurons, and one of the antibodies labeled cells in several other brain regions, including the pars intercerebralis, a known fly sleep center (12, 13) (fig. S4, A and B). The antibody that detected *CycA* in more cells was raised against the whole *CycA* protein, whereas the one that labeled fewer cells was raised against the

CycA N terminus. Both appear to be specific to *CycA*, because their staining was strongly reduced in embryos in which *CycA-RNAi* was driven by a strong, ubiquitous *Gal4* driver (fig. S5). The broader *CycA* expression pattern in the brain was supported by colocalization with *CycA**Gal4*-driven *GFP* (green fluorescent protein), although *GFP* was detected in additional cells in all the clusters (fig. S4A). Overall, *CycA* protein was detected in ~40 to 50 neurons in the brain (fig. S4C).

In the adult fly brain, ~150 clock neurons are organized into six major clusters: three dorsal clusters (DN1, DN2, and DN3), a dorsal-lateral cluster (LNd), and two groups of ventral-lateral neurons—small (s-LNv) and large (l-LNv). The small and large ventral-lateral neurons include neurons that produce the neuropeptide pigment dispersing factor (PDF), a major regulator of clock output (14) (Fig. 4A). s-LNvs are the major pacemaker neurons (15, 16), which also regulate sleep (17). Costaining of brains for *CycA* and PDF revealed that termini of PDF-producing s-LNvs project near the dorsal *CycA* neurons (Fig. 4B). Indeed, as judged by *PDFr**Gal4*-driven *GFP*, which marks presumptive PDF receptor-producing neurons, these *CycA* cells appear to express the PDF receptor (fig. S6A) (18). Such coexpression was found in all *CycA* neurons (examples shown in fig. S6A) and suggests a molecular and neuronal connection between circadian and sleep systems.

Fig. 1. Reduced sleep in animals expressing neuronal *Rca1-RNAi*. **(A)** Pan-neuronal *Rca1-RNAi* [*elav>Rca1-RNAi*, number of animals (*n*) = 235] reduced the duration of daily sleep compared to that of the parental control animals tested as hemizygotes [*elavGal4* (*n* = 121), *UAS-Rca1-RNAi* (*n* = 103)]. Shown are example actograms for *elavGal4* and *elav>Rca1-RNAi* flies that have average or extreme phenotypes. The top actogram for *elav>Rca1-RNAi* shows an example of a fly that is a “normal” sleeper (800+ minutes of sleep), but that did not show consolidation of rest and activity states (phenotype seen in 18 out of 49 “normal” *elav>Rca1-RNAi* sleepers). Dashed lines point from the actograms to the amount of sleep seen in these individual flies. The white and black portions of the bar above the actograms indicate the light and dark periods of the experiment, respectively. **(B)** Comparable activity per waking minute in *elav>Rca1-RNAi* flies and controls. **(C and D)** Decreased sleep bout length (C) and increased number of daily sleep bouts (D) in *elav>Rca1-RNAi* flies, compared to the parental controls. Statistical analysis was performed using one-way analyses of variance (ANOVAs) with Tukey post-test. Shown is statistical significance for experimental genotypes compared to controls with the most similar values. In all figures, error bars represent SEM; in all figures **P* < 0.05, ***P* < 0.01, ****P* < 0.001; n.s., not significant. In all experiments described in this figure, *elavGal4* drives the expression of *UAS-Dicer2*.



Coexpression with *PDFr* also provides an opportunity to knock down CycA abundance in a more limited set of neurons than our pan-neuronal driver had afforded. *PDFrGal4*-driven *CycA-RNAi* resulted in a severe sleep reduction (fig. S6B).

To determine if dorsal CycA neurons are in fact DN1 circadian neurons, we asked whether they express *per*. A subset of the dorsal CycA-containing neurons expressed *perGal4*-driven *GFP* (fig. S6C). A similar partial overlap was seen in all other CycA clusters, with the exception of the pars intercerebralis (fig. S6C). As *perGal4* was expressed in a smaller subset of CycA neurons than *PDFrGal4*, we used the former to knock down CycA in a more limited neuronal subpopulation. In these experiments, sleep was consistently lower than in control animals, although the extent of sleep loss was less extreme than with pan-neuronal CycA knock-

down (fig. S6D). Notably, CycA-containing dorsal neurons that expressed *perGal4* were not stained by an antibody to PER, and thus do not detectably express PER protein. Therefore, dorsal CycA neurons are closely associated with the DN1 cluster (Fig. 4), but do not appear to be clock neurons themselves. A similar relationship exists between CycA-expressing and DN3 or LNV neurons (fig. S7). In contrast, we occasionally found CycA and PER proteins in the same cell within a subset of dorsal-lateral neurons (LNDs), a group of clock neurons that regulate the evening peak of activity (Fig. 4) (15, 16). Finally, a bilaterally symmetric pair of large ventral neurons expressed both CycA and PER proteins (Fig. 4). These ventral neurons do not belong to any known clock neuronal clusters. In summary, CycA-containing neurons are often intermingled with circadian pacemaker neurons. As CycA neu-

rons appear to express the receptor for the circadian neuropeptide PDF, such an arrangement suggests that signals produced by neurons regulating circadian rhythmicity may be directed to neurons that control sleep.

There are other known instances of the repurposing of cell cycle machinery in neurons. Examples include the recent observations that two CDK pathways are essential for proper axonal targeting of presynaptic components in *Caenorhabditis elegans* (19), and that cyclin E regulates synaptic plasticity and memory formation in mice (20). We observed no obvious defects in the gross morphology or number of CycA-containing neurons in *elav>CycA-RNAi* flies and have not detected any obvious rhythmic patterns of CycA protein expression. Nevertheless, such features might become evident in studies focused on specific cells or specific subcellular regions, or in developing or aging

Fig. 2. Reduced sleep in animals expressing neuronal CycA-RNAi. **(A)** Reduced sleep in animals expressing pan-neuronal *CycA-RNAi* [*elav>CycA-RNAi* ($n = 185$)] compared to that of the parental controls [*elavGal4* ($n = 136$), *UAS-CycA-RNAi* ($n = 178$)]. Shown is an actogram of an *elav>CycA-RNAi* animal with an average phenotype. The white and black portions of the bar above the actogram indicate the light and dark periods of the experiment, respectively. **(B)** Normal levels of activity per waking minute in animals expressing *CycA-RNAi* in neurons. **(C and D)** Average sleep bout length was decreased (C), whereas the number of daily sleep bouts was increased (D), in *elav>CycA-RNAi* flies. **(E)** Shorter total sleep of *CycA* null heterozygous flies [*CycA+/-* ($n = 136$)] than that of flies with both functional copies of the gene [*CycA+/+* ($n = 82$)]. Introducing RNAi against either *CycA* or *Rca1* into the *CycA* heterozygous background enhanced the sleep phenotypes [*elav>CycA-RNAi*, *CycA+/-* ($n = 99$); *elav>Rca1-RNAi*, *CycA+/-* ($n = 35$)]. Other controls shown are *UAS-Rca1-RNAi* ($n = 33$), *UAS-CycA-RNAi* ($n = 56$), *elav>CycA-RNAi* ($n = 78$), and *elav>Rca1-RNAi* ($n = 22$). **(F)** Sleep profile of *elav>CycA-RNAi* ($n = 126$), *elav>Rca1-RNAi* ($n = 58$), and control [*elavGal4* ($n = 88$), *UAS-Rca1-RNAi* ($n = 24$), *UAS-CycA-RNAi* ($n = 36$)] flies. Statistical analysis was performed using one-way ANOVAs with Tukey post-test. In all experiments described in this figure, except that shown in (E), *elavGal4* drives the expression of *UAS-Dicer2*.

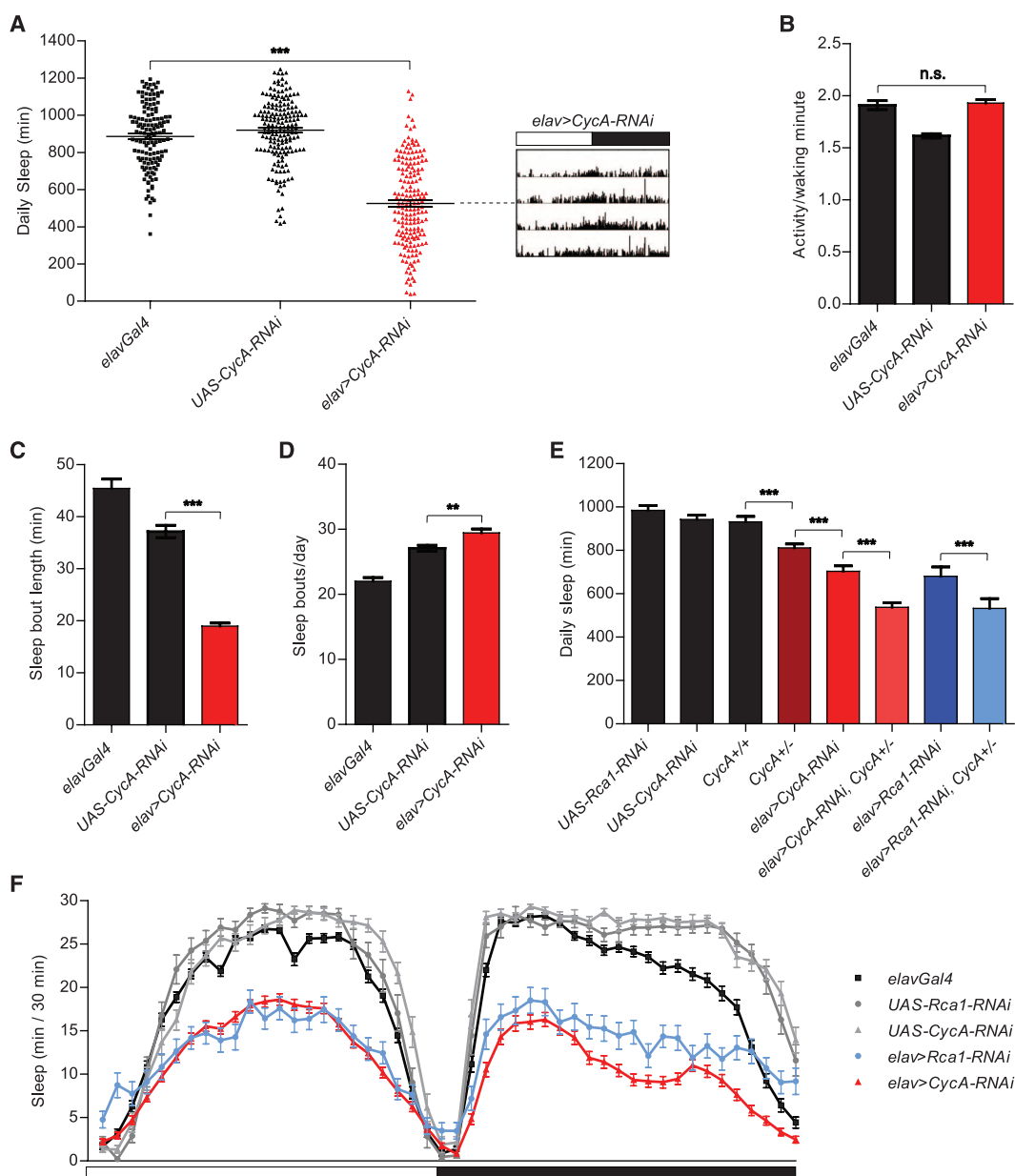


Fig. 3. Reduced homeostasis and decreased life span in *elav>CycA-RNAi* animals. **(A)** Delayed sleep onset in *elav>CycA-RNAi* animals ($n = 80$) compared to controls [*elavGal4* ($n = 64$), *UAS-CycA-RNAi* ($n = 36$)] after lights went off. **(B and C)** Decreased recovery of sleep after overnight sleep deprivation in *elav>CycA-RNAi* animals ($n = 27$), compared to controls [*elavGal4* ($n = 52$), *UAS-CycA-RNAi* ($n = 10$)]. Sleep lost (B) was calculated by subtracting sleep on the night of the deprivation from undisturbed sleep the night before. All animals analyzed lost at least 95% of their sleep. To account for the differences in baseline sleep between the genotypes, recovery data (C) were normalized. **(D)** Reduced life span in *elav>CycA-RNAi* ($n = 113$) animals compared to controls [*elavGal4* ($n = 102$), *UAS-CycA-RNAi* ($n = 100$)]. Statistical analysis of sleep was performed using one-way ANOVAs with Tukey post-test, and statistical analysis of longevity was performed using log-rank tests. In all experiments described in this figure, *elavGal4* drives the expression of *UAS-Dicer2*.

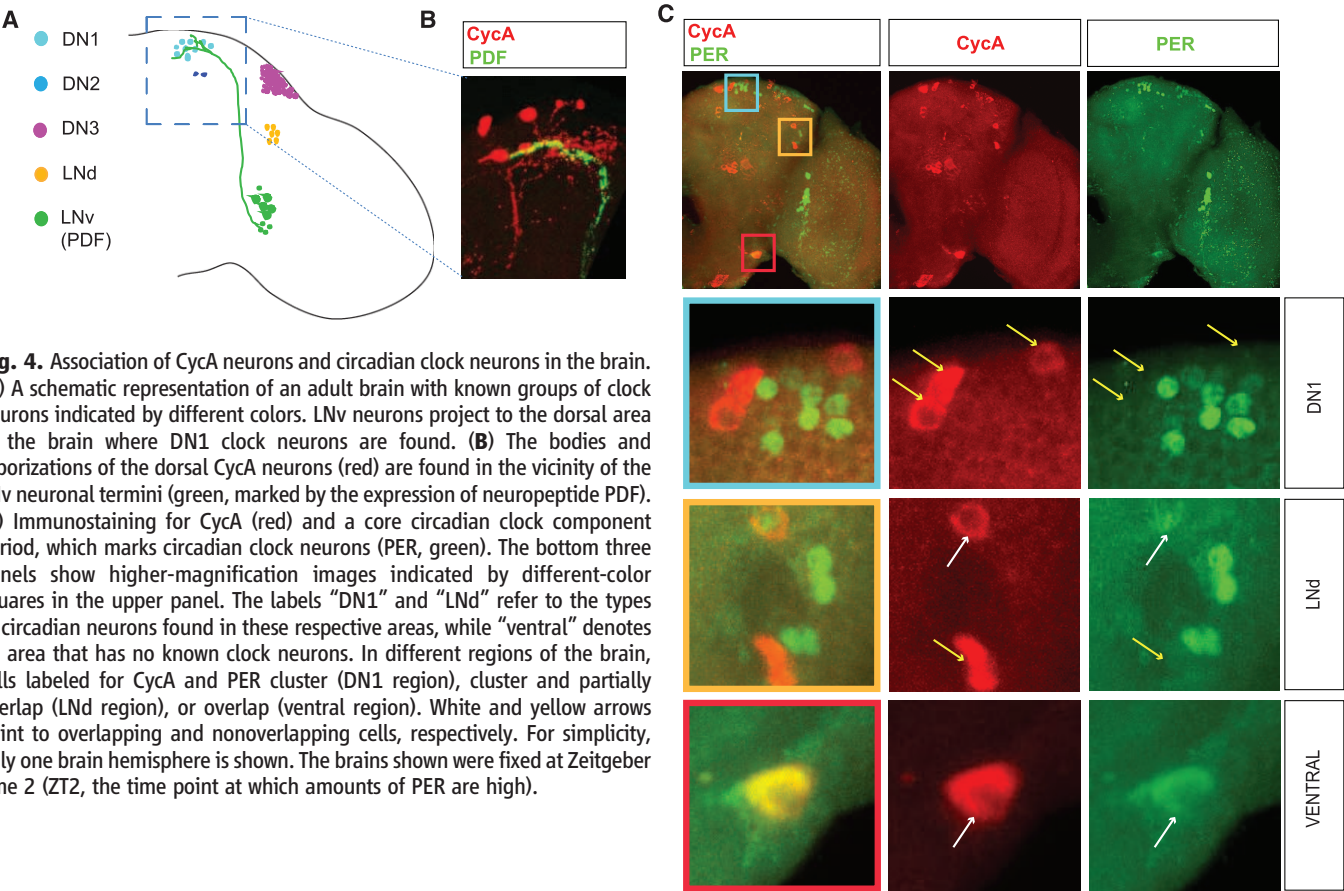
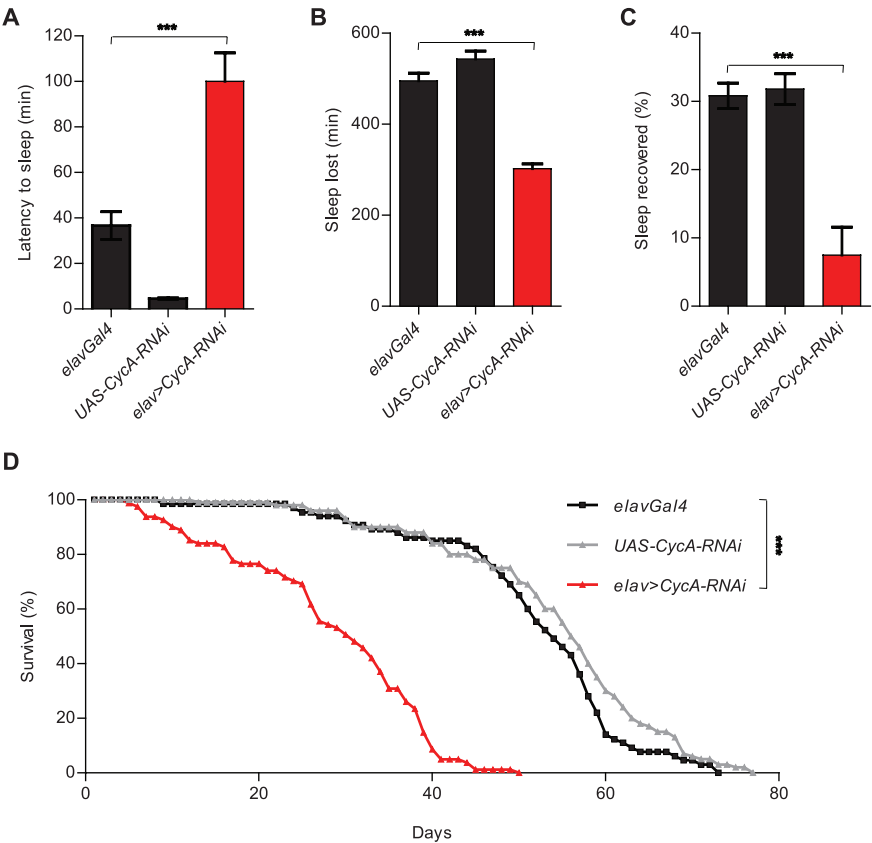


Fig. 4. Association of CycA neurons and circadian clock neurons in the brain. **(A)** A schematic representation of an adult brain with known groups of clock neurons indicated by different colors. LNV neurons project to the dorsal area of the brain where DN1 clock neurons are found. **(B)** The bodies and arborizations of the dorsal CycA neurons (red) are found in the vicinity of the LNV neuronal termini (green, marked by the expression of neuropeptide PDF). **(C)** Immunostaining for CycA (red) and a core circadian clock component Period, which marks circadian clock neurons (PER, green). The bottom three panels show higher-magnification images indicated by different-color squares in the upper panel. The labels “DN1” and “LNd” refer to the types of circadian neurons found in these respective areas, while “ventral” denotes an area that has no known clock neurons. In different regions of the brain, cells labeled for CycA and PER cluster (DN1 region), cluster and partially overlap (LNd region), or overlap (ventral region). White and yellow arrows point to overlapping and nonoverlapping cells, respectively. For simplicity, only one brain hemisphere is shown. The brains shown were fixed at Zeitgeber time 2 (ZT2, the time point at which amounts of PER are high).

flies. Future understanding of the role that CycA plays in determining the structure or activity of neurons specialized for the regulation of sleep seems likely to shed light on the molecular mechanisms underlying this enigmatic behavior. As the role of CycA in cell cycle regulation is conserved throughout metazoans, we can speculate that its role in sleep regulation might be conserved as well.

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Supporting Online Material

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Cotranscriptional Role for *Arabidopsis* DICER-LIKE 4 in Transcription Termination

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Transcription termination is emerging as an important component of gene regulation necessary to partition the genome and minimize transcriptional interference. We have discovered a role for the *Arabidopsis* RNA silencing enzyme DICER-LIKE 4 (DCL4) in transcription termination of an endogenous *Arabidopsis* gene, *FCA*. DCL4 directly associates with *FCA* chromatin in the 3' region and promotes cleavage of the nascent transcript in a domain downstream of the canonical polyA site. In a *dcl4* mutant, the resulting transcriptional read-through triggers an RNA interference-mediated gene silencing of a transgene containing the same 3' region. We conclude that DCL4 promotes transcription termination of the *Arabidopsis* *FCA* gene, reducing the amount of aberrant RNA produced from the locus.

In eukaryotes, termination of RNA polymerase II (Pol II) activity is highly coordinated with RNA 3' processing and cotranscriptional degradation of nascent RNA (1). Cleavage of the nascent transcript at the polyadenylation site produces a entry point for an exoribonuclease, which degrades the Pol II-associated RNA and displaces the Pol II from the template DNA (2, 3). In cases where RNA 3' processing factors fail to cleave at the polyadenylation site, other endoribonucleases supply the exoribonuclease entry point further downstream (4, 5). Dicer proteins are one class of endoribonuclease identified through their production of small RNAs in the size range of 21 to 24 nucleotides (nt). These are often derived from RNA hairpin structures or long double-stranded RNAs (dsRNAs) from repeated sequences and multicopy transposons (6). In *Arabidopsis*, four different DICER-LIKE proteins (DCL1 to 4) have been associated with

gene-silencing mechanisms via microRNA, small interfering RNA (siRNA), and trans-acting siRNA (tasiRNA) action (7). However, it is becoming clear that Dicer-dependent mechanisms are also important for regulation of endogenous single-copy genes (8, 9).

The nuclear-localized RNA binding protein *FCA* induces chromatin silencing of the *Arabidopsis* flowering-time regulator *FLOWERING LOCUS C* (*FLC*). *FCA* alters poly (A) site usage of *FLC* antisense transcripts, which, in turn, induces H3K4me2 demethylase activity and reduced *FLC* expression (10, 11). In a suppressor screen (using a genotype overexpressing *FCA*), we identified a mutant (*sof59*) that increases levels of *FLC* (Fig. 1A) through reduction of expression of the 35S::*FCAγ* transgene (Fig. 1B) (12). We found the causative mutation to be a premature stop codon in *DCL4* (Fig. 1C). Complementation of the mutant with a wild-type (WT) *DCL4* transgene fully restored expression of 35S::*FCAγ* (Fig. 1B). We named this mutant allele of *DCL4* in *Ler* (*Landsberg erecta*) background as *dcl4-15*.

In *Arabidopsis*, DCL4 is required for the biogenesis of tasiRNAs. tasiRNA generation in-

volves RNA DEPENDENT RNA POLYMERASE 6 (RDR6) and SUPPRESSOR OF GENE SILENCING 3 (SGS3) (13, 14). Expression of the 35S::*FCAγ* transgene was not affected by *rdr6* or *sgs3* mutations (fig. S1), arguing that reduced expression of the 35S::*FCAγ* transgene in *dcl4* was not due to loss of tasiRNA or related developmental defects.

The requirement of DCL4 to maintain expression of the 35S::*FCAγ* transgene runs counter to its role in gene silencing. The 35S::*FCAγ* transgene is composed of the strong 35S promoter, *FCAγ* cDNA, and ~420 base pairs (bp) of the 3' region from the endogenous *FCA* gene as the 3' termination region (fig. S2) (15). We detected polyadenylated read-through transcripts from this transgene (Fig. 1B); they either terminated in the vector sequence 861 or 947 nt downstream of the major canonical *FCA* polyadenylation site or were spliced and terminated a further 1000 nt downstream, within the terminator sequence of the convergently transcribed adjacent kanamycin-resistance transgene *NPTII* (fig. S2). These data suggested that the *FCA* 3' sequences terminate transcription inefficiently.

We also observed transcriptional read-through for the endogenous *FCA* gene in multiple *dcl4* mutant alleles: *dcl4-15*, *dcl4-2t*, *dcl4-3*, and *dcl4-4*, but not in the weak allele *dcl4-6* (Fig. 2B and figs. S3 to S5) (16), and this was associated with increased Pol II occupancy in this region (Fig. 2C). Transcription termination within *Schizosaccharomyces pombe* centromeric repeats also depends on Dicer function (17). To further confirm a direct role for DCL4 in transcription termination, we performed nuclear run-on experiments. This analysis showed a clear increase in transcriptional read-through into the region downstream of the *FCA* polyadenylation site in *dcl4* compared with the wild type (Fig. 2D). The read-through transcripts overlap with the adjacent convergently transcribed gene At4g16270 (fig. S3). This overlapping convergent transcription did not seem to trigger a sense/antisense pairing and destabilization mechanism as quantitative strand-specific reverse transcription polymerase chain reaction (qRT-PCR)

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showed that expression of At4g16270 did not change in *dcl4* (fig. S3). In addition, no 21-nt siRNA could be detected using high-sensitivity Northern analysis. These data suggest that DCL4 functions to repress accumulation of endogenous *FCA* read-through transcripts in a strand-specific manner.

Using chromatin immunoprecipitation (ChIP) analysis, we found that DCL4 associates directly (an enrichment of ~threefold) with endogenous *FCA* chromatin in a region 600 to 1200 bp downstream of the polyadenylation site (Fig. 2E and fig. S6). DCL4 was not detected in the body of the *FCA* gene (Fig. 2E and fig. S6) or in the 3' region of *FLC* or *ACTIN* (fig. S6), and transcriptional read-through at these loci was not affected by *dcl4* (fig. S7). Thus, DCL4 functions cotranscriptionally to repress transcriptional read-

through at endogenous *FCA* but is not an absolute requirement for transcription termination over the whole *Arabidopsis* genome.

Rnt 1, a dsRNA-specific ribonuclease III, mediates cotranscriptional cleavage (CoTC) of nascent RNA downstream of the polyadenylation site in *Saccharomyces cerevisiae* (5). The domain structure of Rnt 1 is very similar to the C terminus of DCL4—an RNase III domain followed by a dsRNA binding domain. To investigate if DCL4 could be involved in repressing read-through of endogenous *FCA* through a similar mechanism, we mapped the 3' ends of the *FCA* read-through RNA. To do this, we used hybrid selection circular rapid amplification of cDNA ends, a technique that enables read-through transcripts to be specifically enriched (fig. S8) (18). In Ler WT plants, the majority of the read-through products ended in the region 122 to 561 nt downstream of the *FCA* polyadenylation site, with most (66 to 84%) between 230 to 390 nt (Fig. 3 and fig. S9). In *dcl4-15*, no read-through transcripts terminated in the 230- to 390-nt window, and more transcripts ended further downstream compared with the wild type (Fig. 3 and fig. S9). This DCL4-dependent cleavage site was at one end of the domain of DCL4 association defined by ChIP (Fig. 2E). DCL4 might recognize a secondary RNA structure (19), which can be formed by the nascent transcript read-through product (fig. S10). In human cells, a very short

polyA tail is added to the 3' end of the CoTC product followed by exosome-mediated trimming (18). However, the mapped 3' ends of read-through products of endogenous *FCA* did not contain short A tails, so may represent trimmed products following a DCL4-dependent CoTC activity. These data suggest that DCL4 enhances transcription termination of endogenous *FCA* by facilitating CoTC of the nascent RNA downstream of the polyadenylation site.

The transgene does not contain the sequences to which DCL4 associated, and, consistent with this, no DCL4 enrichment was detected in the 3' region of the transgene (fig. S11). Therefore, we hypothesized that it was the read-through transcription of the endogenous *FCA* gene that triggered the transgene silencing. Supporting this model, a transferred DNA (T-DNA) insertion blocking the endogenous *FCA* transcriptional read-through (fig. S12) attenuates the transgene silencing in *dcl4* (Fig. 4A). The mechanism of transgene silencing appears to involve both Dicer-like 2 and 3 (DCL2 and DCL3), as revealed through accumulation of 22- and 24-nt small RNAs homologous to the transgene in *dcl4-15* (Fig. 4B). A *dcl2*, *dcl4* double mutant fully restored expression of the 35S::FCA γ transgene (Fig. 4C), removed the 22-nt small RNAs (Fig. 4D), and prevented RDR6-dependent copying of sense RNA (fig. S13). These data suggest that a DCL2-dependent posttranscriptional gene

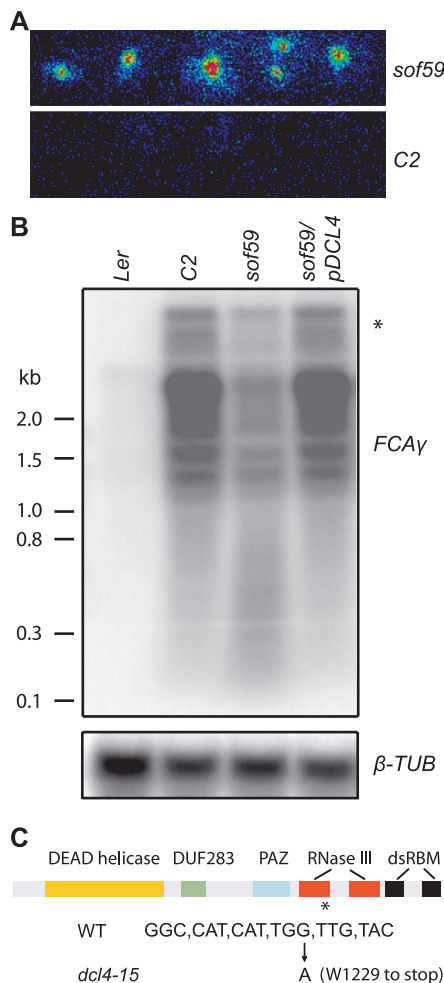
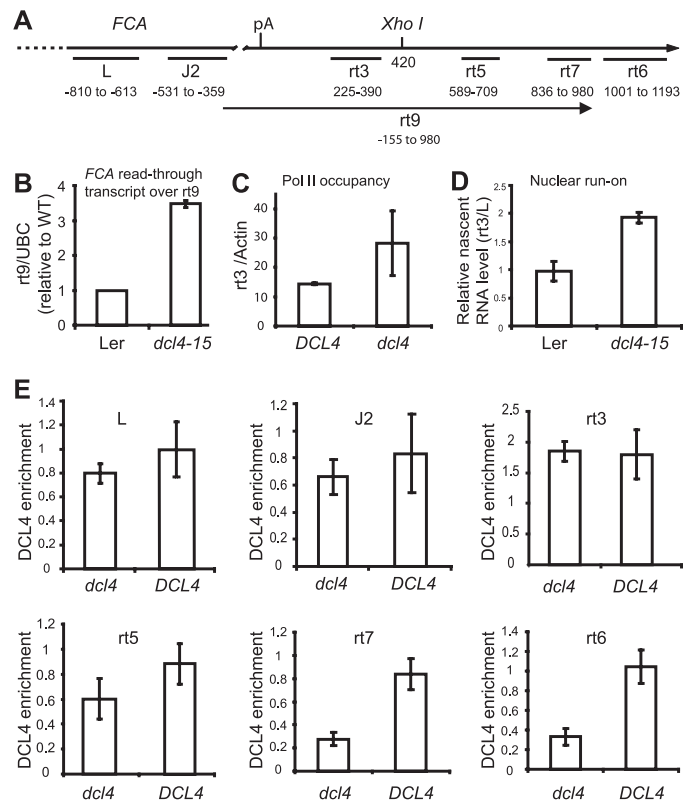


Fig. 1. Identification of DCL4 as a regulator of *FCA* expression. (A) *FLC::LUC* signal in *sof59* and its parental line C2. (B) Expression of 35S::FCA γ transgene is reduced in *sof59* and is rescued by reintroduction of DCL4. Total seedling RNA hybridized with single-stranded antisense probe from 35S::FCA γ . The asterisk indicates read-through transcripts. Blots reprobed with β -TUBULIN (β -TUB) are shown as loading control. (C) Schematic representation of DCL4 and the position of the mutation.

Fig. 2. Transcriptional read-through of endogenous *FCA* and association of DCL4 with the *FCA* 3' region. (A) Location of PCR fragments in the 3' region of *FCA* assayed in (B) to (E). pA indicates an *FCA* polyadenylation site; numbers under each fragment indicate the nucleotide distance from *FCA* pA. (B) Semi-qRT-PCR over rt9 analyzing *FCA* sense read-through RNA normalized to *UBC* (ubiquitin conjugating enzyme At5G25760). Data are normalized to the Ler wild type. (C) Pol II ChIP enrichment (IP/input) over rt3 normalized to that of actin (At5g09810). (D) Transcripts from nuclear run-on experiments over the rt3 region normalized to the L region. (E) DCL4 ChIP assayed by qPCR over the *FCA* gene body (L and J2) and 3' region (rt3, rt5, rt7, rt6). Enrichment (IP/input) is normalized to that of *STM* (At1g62360). Values in (B) to (E) are means $\pm$ SEM; $n = 3$ biological replicates.



silencing is the predominant silencing mechanism (20, 21). A minor role for DCL3 in silencing the 35S::FCAy transgene was indicated by the partial restoration of expression in a *dcl3*, *dcl4* double mutant (Fig. 4, C and D, and fig. S13). The 24-nt small RNAs also induced DNA

and H3K9 methylation on the 35S::FCAy transgene (figs. S14 and S15).

We conclude that DCL4 can promote expression of endogenous genes by contributing to the “tidying up” of 3′ end formation at genes where, for some reason, effective termination

and polyadenylation has not occurred (fig. S16). The relatively weak canonical polyadenylation at the endogenous *FCA* 3′ region would lead to read-through RNA that potentially folds into structures recognized by DCL4. DCL4 would function as a CoTC enzyme enhancing transcription termination. Loss of this activity would increase levels of aberrant RNA from the endogenous gene. In the presence of high levels of *FCA* RNA from the 35S::FCAy transgene, the aberrant RNA from the endogenous gene would efficiently trigger robust RNAi-mediated gene silencing (20–22). Our work has thus revealed a previously unrecognized cotranscriptional function for *Arabidopsis* DCL4 in transcription termination of an endogenous gene.

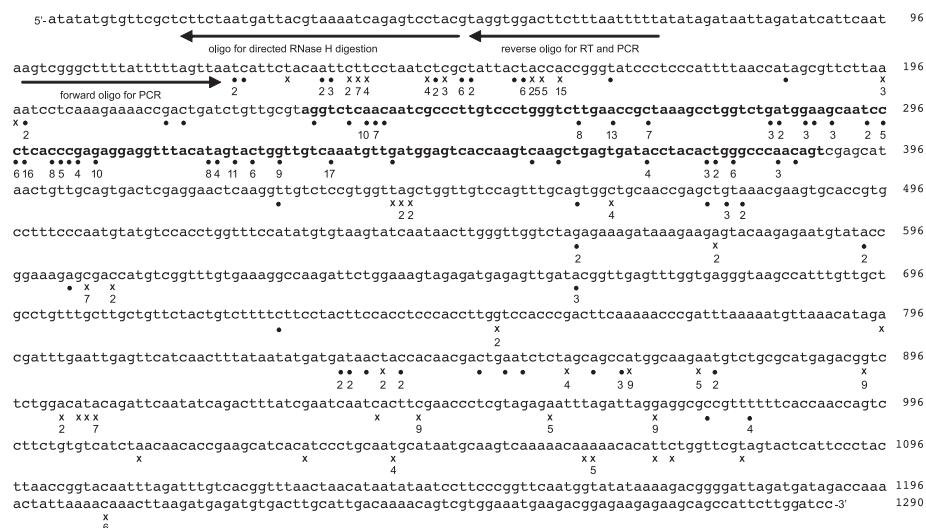
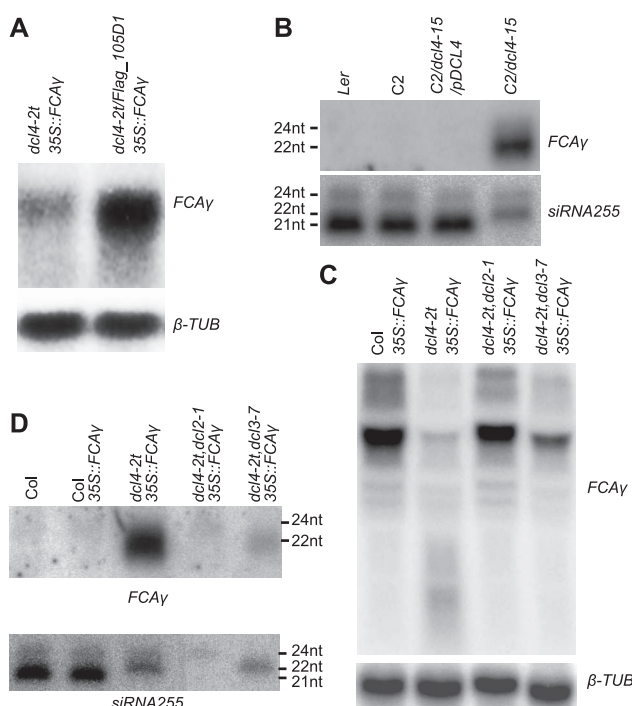


Fig. 3. 3′ ends of endogenous *FCA* read-through transcripts in the wild type and *dcl4*. The sequence shown here is the endogenous *FCA* gene starting downstream of the polyadenylation site. Numbers on the right of each line indicate the nucleotide position relative to pA. The biotin-labeled antisense probe used to affinity purify the *FCA* transcripts was upstream of the sequence shown (−375 to −234 relative to the pA site). The sequence used for oligonucleotides for RNase H digestion, RT and PCR reactions is indicated. The mapped 3′ ends of the *FCA* transcripts are shown below the sequence. Dots indicate 3′ ends from the Ler wild type, whereas crosses indicate 3′ ends from *dcl4*-15. The numbers under the arrows indicate number of clones; symbols without a number indicate a single clone. The sequence to which most of the 3′ ends mapped in the Ler wild type is shown in bold. The data are combined from three biological repeats. Analysis of same data set with individual biological repeats is shown in fig. S9.

Fig. 4. Read-through transcripts from endogenous *FCA* trigger an RNAi-mediated silencing of the 35S::FCAy transgene in a *dcl4* background. (A) A T-DNA insertion (Flag_105D1) that prevents read-through from endogenous *FCA* attenuates the 35S::FCAy transgene silencing. (B and D) Small RNA blots hybridized with dsDNA probes over the 35S::FCAy coding region. The blots were reprobed with an antisense oligonucleotide against siRNA255 (a tasRNA). (C) Total RNA from seedlings of different genotypes was hybridized with a single-stranded antisense probe of 35S::FCAy. The blots were reprobed with β -TUBULIN (β -TUB) as a loading control. Col (Columbia) is the cognate wild type of *dcl4*-2t.



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Supporting Online Material

www.sciencemag.org/cgi/content/full/335/6076/1621/DC1
Materials and Methods
Figs. S1 to S16
References (23–25)

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Gap Junctions Compensate for Sublinear Dendritic Integration in an Inhibitory Network

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Electrically coupled inhibitory interneurons dynamically control network excitability, yet little is known about how chemical and electrical synapses regulate their activity. Using two-photon glutamate uncaging and dendritic patch-clamp recordings, we found that the dendrites of cerebellar Golgi interneurons acted as passive cables. They conferred distance-dependent sublinear synaptic integration and weakened distal excitatory inputs. Gap junctions were present at a higher density on distal dendrites and contributed substantially to membrane conductance. Depolarization of one Golgi cell increased firing in its neighbors, and inclusion of dendritic gap junctions in interneuron network models enabled distal excitatory synapses to drive network activity more effectively. Our results suggest that dendritic gap junctions counteract sublinear dendritic integration by enabling excitatory synaptic charge to spread into the dendrites of neighboring inhibitory interneurons.

Inhibitory interneurons balance network excitation, enhance spike-time precision of principal neurons, and control synchrony within and across brain regions (1–4). Yet, little is known

about how interneurons integrate chemical (2, 4–6) and electrical (7) synaptic inputs because of the inaccessibility of their dendrites. To investigate this, we studied dendritic integration in Golgi cells (GoCs), the main inhibitory interneurons in the input layer of the cerebellar cortex. GoCs receive excitatory mossy fiber (MF) (8) and parallel fiber (PF) (9) inputs onto their proximal and distal dendrites, respectively, and are predominantly interconnected by gap junctions (GJs) (10, 11). Two-photon uncaging of 4-methoxy-7-

nitroindolyl glutamate (12) was used to mimic synaptic inputs at different dendritic locations (Fig. 1A). We tested the linearity of synaptic integration in a dendritic branch by comparing the arithmetic sum of individual photolysis-evoked excitatory postsynaptic potentials (pEPSPs), generated at different locations (section range 10 to 35 μm ; $18 \pm 5 \mu\text{m}$, 49 cells), with the response when all locations were activated synchronously (within a 3-ms window). For small pEPSPs, the synchronous response and arithmetic sum were similar, but for stronger pEPSPs, the integration became markedly sublinear (Fig. 1B). Sublinear integration was observed in apical and basolateral dendrites (0 to 261 μm from soma, $120 \pm 54 \mu\text{m}$, 65 apical and 16 basolateral branches) (fig. S1, A and B). However, for a given size of pEPSP recorded at the soma, sublinear dendritic integration became more pronounced with increasing distance from the soma (Fig. 1, C and D, and fig. S1C).

To investigate whether active conductances are involved in sublinear dendritic integration, we made paired whole-cell recordings from the dendrite and soma using two-photon guided patching (13) (Fig. 2A). Although GoC dendrites are thin (diameter $0.9 \pm 0.1 \mu\text{m}$, $n = 19$), recordings could be made up to 184 μm from the soma (range 19 to 184 μm ; $88 \pm 47 \mu\text{m}$). Resting potentials at the soma and dendrite were similar ($P > 0.05$) [see supporting online material (SOM)]. Current injections into the dendrite produced voltage

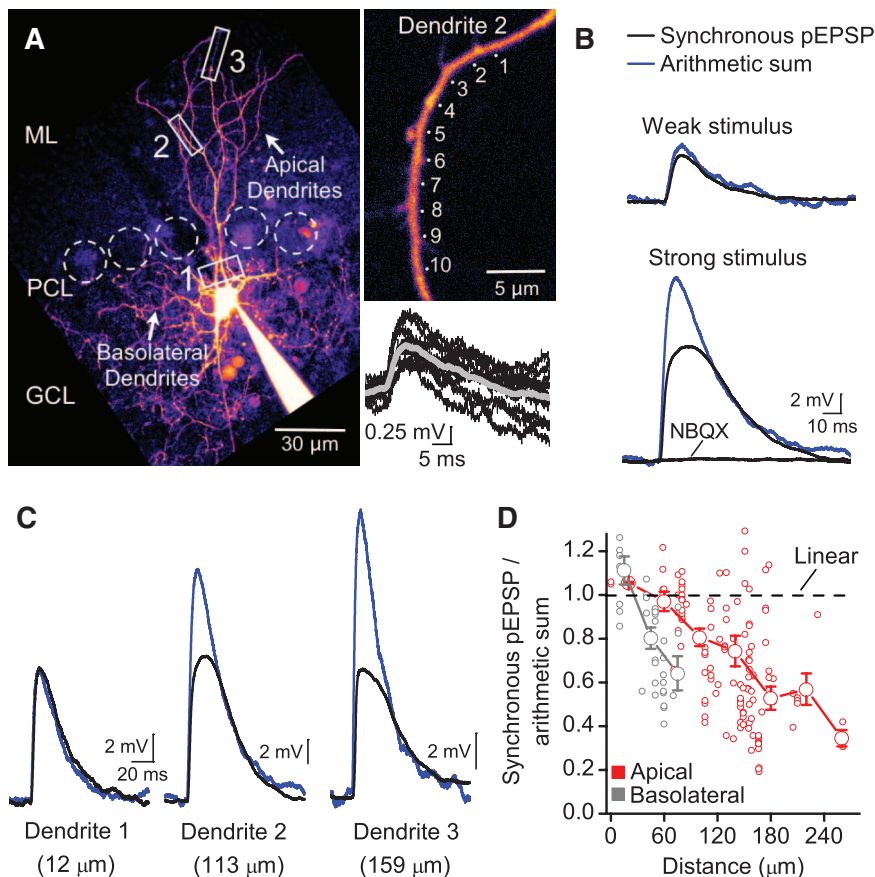


Fig. 1. Distance-dependent sublinear dendritic integration in GoCs. **(A)** (Left) GoC filled with Alexa-594 dye; granule cell layer (GCL), Purkinje cell layer (PCL) (dashed circles), and molecular layer (ML). (Right) Glutamate uncaging locations for dendrite 2 on left and individual pEPSPs at about -70 mV for 10 locations; average in gray. **(B)** (Top) Mean arithmetic sum of individual pEPSPs (five locations) and mean pEPSP evoked by synchronous uncaging at the same locations. (Bottom) Uncaging at 10 locations and the effect of 10 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide (NBQX). **(C)** Synchronous pEPSP and arithmetic sum for each dendritic branch indicated in **(A)**; number of uncaging locations and laser power were adjusted to evoke similar somatic pEPSP amplitudes. **(D)** Ratio of synchronous pEPSP amplitude to arithmetic sum versus branch-soma distance. Only synchronous pEPSPs with similar amplitude (3 to 5 mV) were used (averaged over 40- μm bins).

changes that were maintained for the duration of the pulse (distance $116 \pm 37 \mu\text{m}$, eight cells) (Fig. 2B) and had a linear current-voltage relation [correlation coefficient (r) = 0.99] (Fig. 2C). In contrast, somatic current injections produced hyperpolarizing voltage responses with a sag and a nonlinear current-voltage relation [recorded in tetrodotoxin (TTX)] (Fig. 2, B and C). Injection of artificial excitatory postsynaptic current (EPSC)-shaped currents (aEPSCs) (fig. S2) into the dendrites produced EPSP-like local voltage responses (distance $103 \pm 28 \mu\text{m}$, five cells) (Fig. 2D). The waveform of these aEPSPs was invariant, irrespective of the size of the depolarization (Fig. 2D, inset, and fig. S3, A to C), which indicated linear dendritic behavior. In contrast, aEPSC injections into the soma produced aEPSPs with decays that accelerated with depolarization (eight

cells, in TTX) (Fig. 2E), which indicated the presence of perisomatic active conductances. The integrals of the dendritic and somatic responses were markedly different (Fig. 2F). Cell-attached recordings from the soma confirmed the presence of depolarization-activated outward currents (12 patches), consistent with K^+ conductances in GoCs (9, 14), but negligible inward or outward currents were observed in dendritic patches (15 patches) (Fig. 2, G and H). Any functional voltage-activated Na^+ , Ca^{2+} , and K^+ channels present on the dendrite are, therefore, below our detection limit and do not contribute substantially to the electrical properties of GoCs. Seven additional observations confirmed that few intrinsic voltage-gated channels are present on GoC dendrites: (i) the K^+ channel antagonists tetraethylammonium and 4-aminopyridine had no effect on pEPSPs (fig.

S3, D and E); (ii) the SK-channel blocker apamin had no effect on PF EPSPs (fig. S4); (iii) there was little or no hyperpolarization-activated current (h current)-dependent sag (9) in dendritic recordings (Fig. 2B and fig. S5A) or in somatic recordings when the axon was cut (fig. S5B); (iv) immunolabeling for the HCN1 subunit, which mediates the h current, was strong in the axon, but no detectable immunoreactivity was found in dendrites (fig. S5, C to E); (v) differentiating the pEPSP rise showed no evidence of dendritic spikes (12) (fig. S1, D to G); (vi) the persistent Na^+ current was blocked by local TTX application to the axon initial segment (AIS) (fig. S6); and (vii) immunolabeling for Na^+ channel subunits was strong in the AIS but not detectable in GoC dendrites (fig. S7, E and F).

Distal synapses can have a larger conductance than proximal inputs (15). To examine whether this counteracts dendritic attenuation in GoCs, we made dual soma-dendrite recordings and compared the properties of fast-rising spontaneous EPSPs (sEPSPs), arising close to the dendritic pipette (13, 16), and aEPSPs (15). The amplitude of dendritic sEPSPs increased with distance from the soma, whereas their amplitude at the soma decreased (Fig. 3, A and B). The effective space constants for dendrite-to-soma attenuation of sEPSPs and aEPSPs were comparable (Fig. 3C), which suggests that synaptic conductance does not compensate for dendritic attenuation. *N*-Methyl-D-aspartate (NMDA) receptors made only a small contribution to PF responses (fig. S8, A to C), similar to that observed for MF inputs (8).

For some inhibitory interneurons, the axon arises from the dendrite (2, 5, 6) rather than from the soma. Because this affects how synaptic inputs are integrated, we made paired soma-dendrite recordings to determine the spike initiation site in GoCs. Current injection into the dendrite or soma evoked an action potential (AP) that always occurred first at the soma ($n = 19$ cells, $r = 0.83$; $P < 0.001$) (Fig. 3, D and E). Moreover, the rapid decay of the AP amplitude with distance from the soma (Fig. 3F) is consistent with a passive spread of voltage into the dendrites (5, 6). To distinguish whether the AP arose in the soma or axon, we made whole-cell recordings from putative axons (29 to 57 μm from soma, $43 \pm 10 \mu\text{m}$, $n = 6$) (fig. S7, A to C). Current injections into the axon or soma triggered APs that always occurred first in the axon. Immunolabeling with the GoC-specific marker metabotropic glutamate receptor 2 (mGluR2) (17) and the AIS markers ankyrin-G, pan- Na_v , or $\text{Na}_v1.6$ confirmed that a single AIS arose $3.7 \pm 1.5 \mu\text{m}$ ($n = 12$) from the soma and that it expressed a high density of Na^+ channels (fig. S7, D to F).

In vivo, GoCs receive both synchronous (18) and slowly modulated asynchronous synaptic inputs (19) that are spatially distributed on the dendritic tree. Because it is difficult to study this experimentally, we developed experimentally constrained multicompartment models of GoCs that matched our measurements (Fig. 3, C, E, and F)

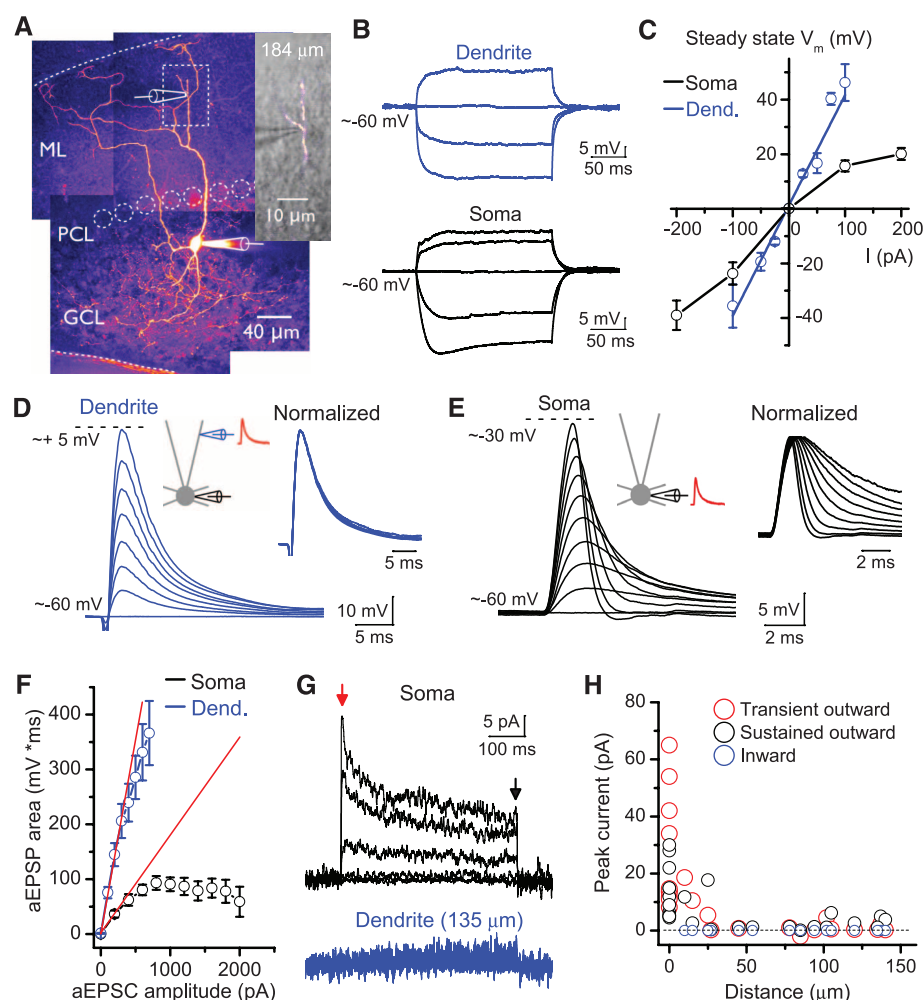


Fig. 2. GoC dendrites behave as linear cables. (A) GoC filled with Alexa-594 dye and pipette locations. (Inset) Superimposed DAPI-contrast and fluorescence images of the patched dendrite. (B) (Top) Dendritic voltage responses to dendritic current pulses of equal magnitude. (Bottom) Somatic voltage responses to somatic current pulses (in 1 μM TTX). (C) Steady-state current-voltage relations. (D) Dendritic voltage responses to dendritically injected aEPSCs (red) of increasing amplitude. (Inset) Seven responses normalized to peak. (E) Somatic voltage responses to somatic aEPSCs in 1 μM TTX. (Inset) Nine responses normalized to peak. (F) Relation between aEPSP area and aEPSC amplitude, with linear extrapolations to the initial two responses. (G) Cell-attached voltage-clamp recordings; current responses to 20-mV steps from -80 mV to $+40$ mV. (H) Amplitude of transient outward currents (red arrow in G), sustained outward currents (black arrow in G), and inward currents versus distance to soma.

and reproduced a wide range of firing behavior (10, 20). Synaptic inputs with identical conductance waveforms (fig. S2D) were randomly distributed over the dendritic tree: Those in the granule cell layer were defined as MFs, whereas those in the molecular layer were defined as PFs (Fig. 3G, inset). Synchronous activation of subsets of six randomly selected MF inputs was required to fire the model GoC from -60 mV (Fig.

3G), as found experimentally (8). In contrast, 11 PF inputs were required to fire the model cell, which demonstrated that distal inputs are less efficient (Fig. 3G). Sustained asynchronous MF inputs were also more effective than PF inputs, as they produced a higher firing rate (Fig. 3H). Similar results were obtained for two other morphologically reconstructed GoCs (fig. S9). During asynchronous PF input, dendrites in the

molecular layer depolarized more than dendrites in the granule cell layer during MF input, because the latter were more hyperpolarized by perisomatic pacemaker conductances (9, 14, 20) (Fig. 3I). The reduced driving force in distal dendrites limited the synaptic current generated, which made PFs less effective than MFs and explained the distance-dependent sublinear summation of pEPSPs (Fig. 1). Indeed, converting synaptic

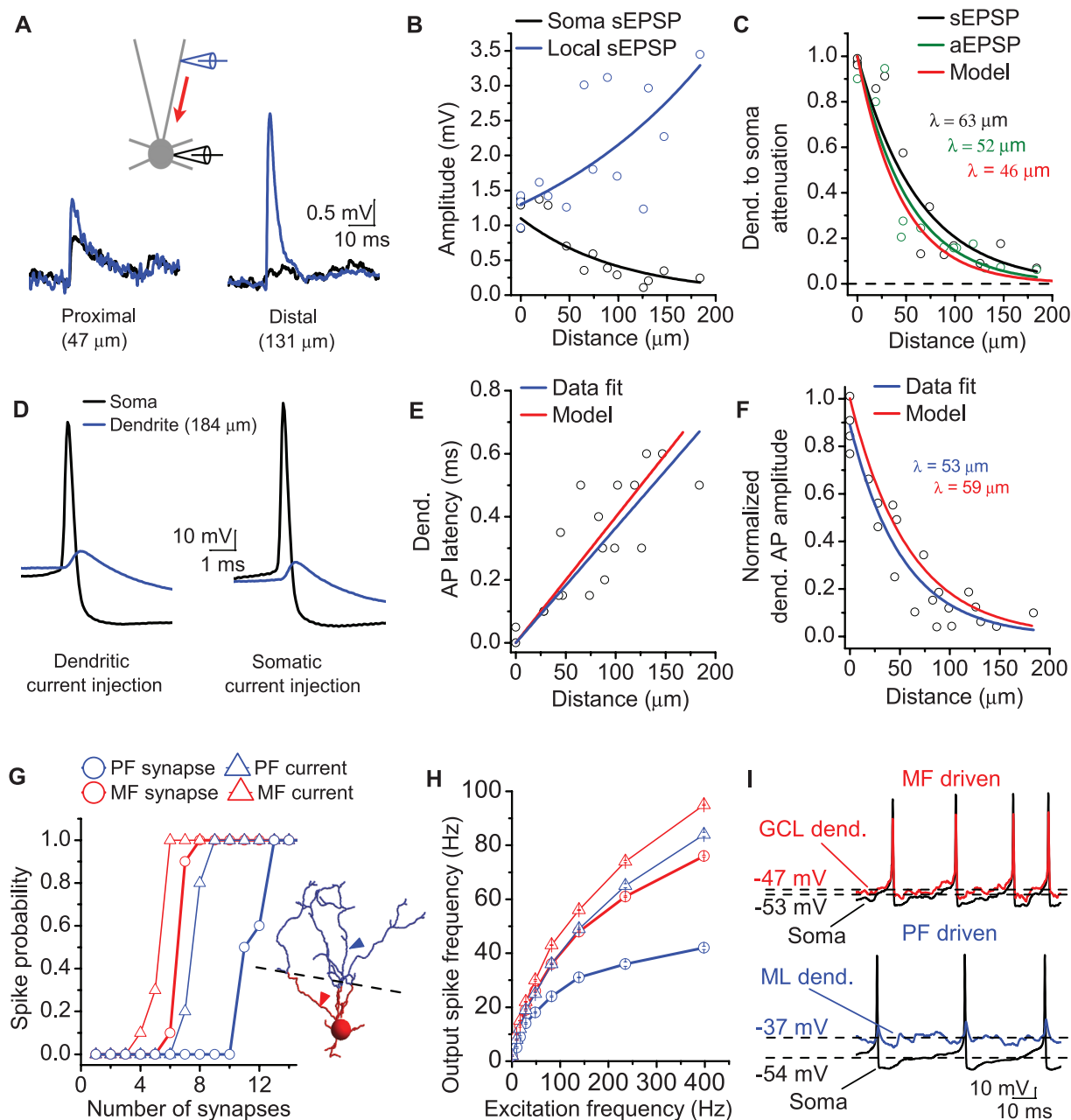


Fig. 3. Passive dendrites weaken distal synaptic inputs. **(A)** Simultaneous somatodendritic recordings of fast-rising (<0.5 ms) sEPSPs originating locally in the dendrite. Pipette locations are shown. **(B)** Amplitudes of sEPSPs in the dendrite and soma versus soma-dendrite distance. **(C)** sEPSP and aEPSP attenuation from dendrite to soma, with exponential fits and model prediction from morphologically realistic GoC model. **(D)** Simultaneously recorded APs in soma and dendrite, evoked by current injection (50 pA, 200 ms). **(E)** Latency between somatic and dendritic APs. **(F)** Attenuation of back-propagating APs

with soma-dendrite distance. **(G)** (Inset) Reconstructed GoC morphology for modeling [red, granule cell layer (GCL), blue, molecular layer (ML)]. (Main) Relations between spike probability and number of inputs for synchronous MF and PF synaptic conductance and current (10 simulations). **(H)** Mean input-output relations for asynchronous MF and PF synaptic inputs (four simulations). **(I)** Simulated somatic and dendritic voltage traces for granule cell layer and molecular layer dendrites [arrowheads in (G) indicate locations] during MF and PF inputs (at ~ 230 Hz).

conductances to currents (which are independent of driving force) in our model reduced the difference in efficacy between MFs and PFs during both synchronous and asynchronous input (Fig. 3, G and H).

As for many types of inhibitory interneurons in the brain (7), GoCs are coupled through electrical synapses, mediated by GJs composed of connexin-36 (Cx36) subunits (10, 11). We examined their impact on input resistance (R_{input})

by applying 20 μM mefloquine, a Cx36-specific concentration that blocks $\sim 70\%$ of the GJ conductance (21). This increased R_{input} in wild-type mice by 48% ($P = 0.0002$; $n = 13$ cells), without having an effect on GoCs from Cx36 knockout mice (22) ($P = 0.2$) (Fig. 4A). This difference was significant ($P = 0.01$). Moreover, GoCs from Cx36 knockout mice ($n = 8$) had a 77% higher R_{input} than those from wild-type mice ($P = 0.007$, $n = 7$) (Fig. 4A). To examine how GJs are distributed, we counted Cx36-positive puncta on mGluR2-positive GoCs. This revealed a non-uniform GJ density, which peaked in the dendrites located in the inner molecular layer (Fig. 4B) and provided no evidence of axonal GJs (fig. S10). Consistent with this distribution, cutting off the molecular layer dendrites markedly reduced the effect of mefloquine on R_{input} ($P = 0.003$, $n = 5$) (Fig. 4A and fig. S11). Using the density of Cx36 puncta and mean dendritic surface area from reconstructions ($n = 5$), we estimate that a GoC forms 35 GJs.

To explore how electrical synapses and passive dendritic properties affect chemical synaptic integration at the network level, we built experimentally constrained GoC network models (10, 23) (Fig. 4C). Electrical coupling was implemented probabilistically, with discrete GJ conductances, using the nonuniform GJ density (Fig. 4B) and distance-dependent (soma-soma) coupling probabilities (10) (see SOM). Removing GJs from the models increased GoC R_{input} , as found experimentally (Fig. 4A). Without GJ coupling, network models exhibited different mean input-output relations for MF-only and PF-only excitation (Fig. 4D), as for the single GoC models. By contrast, when GJs were included, synaptic excitation was enhanced, and the difference between MF- and PF-driven network excitation was reduced (Fig. 4D). Separate analysis of innervated and noninnervated GoCs (Fig. 4, E and F) revealed GJ-mediated excitation of the latter population. We tested this model prediction with GoC paired recordings. These showed that depolarization of a single GoC could “wake up” neighboring GoCs and cause them to fire APs (four pairs) (Fig. 4G). Simulations revealed that the efficacy with which MFs and PFs excite non-innervated GJ-coupled cells is switched compared with the efficacy of excitation of innervated cells (Fig. 4, E and F). Moreover, inclusion of synaptic NMDAR components further enhanced the efficacy of PFs over MFs in driving noninnervated GJ-coupled cells (fig. S8, F and I). Two factors augmented the spread of excitatory charge from PF synapses to neighboring GoC dendrites: colocalization of PF synapses and GJs in the molecular layer minimized dendritic attenuation of current (Fig. 4H), and GJ driving force was larger because distal dendrites depolarized more during synaptic input (Fig. 3, A and I). GJ-mediated enhancement of synaptic excitation was robust across a wide range of GJ conductances and densities (Fig. 4I and fig. S12), was robust in the presence of chemical inhibitory conductances,

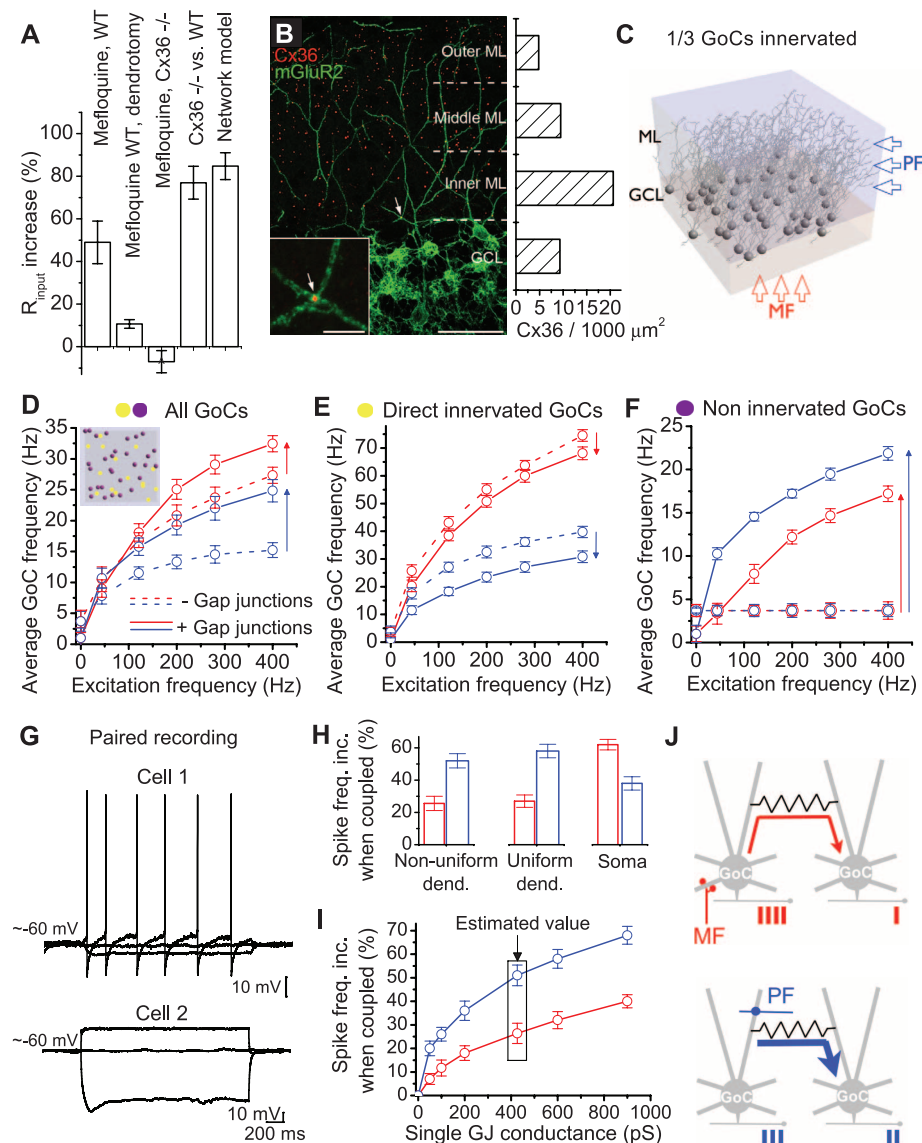


Fig. 4. Dendritic gap junctions enhance the efficacy of distal synaptic input. **(A)** (Left to right) Mean ($\pm$ SEM) increase in GoC input resistance (R_{input}) with 20 μM mefloquine for wild-type (WT), WT dendotomy, and Cx36^{-/-} mice, respectively [in 10 μM NBQX, 50 μM D(-)-2-amino-5-phosphonopentanoic acid (AP5), and 10 μM SR95531]; between Cx36^{-/-} mice and Cx36^{+/+} littermates; and when GJs were removed from GoC network models. **(B)** Cx36 immunopuncta (red) on mGluR2-positive dendrites (green) in the granule cell layer (GCL) and the inner-, middle-, and outer molecular layer (ML). Scale bar, 50 μm . (Inset) Cx36 punctum between two mGluR2-positive dendrites, Scale bar, 5 μm . Bar graph indicates density of Cx36 puncta. **(C)** Network model with 45 GoCs; 15 were innervated by MF or PF inputs. **(D)** Network input-output relations showing the mean spike frequency across all GoCs during asynchronous MF (red) or PF input (blue) without (dashed lines) and with (solid lines) GJs between cells (mean $\pm$ SD, four networks). (Inset) Top view showing innervated (yellow) and noninnervated (purple) GoCs. **(E)** and **(F)** Input-output relations for innervated (**E**) and noninnervated (**F**) GoCs only. **(G)** Voltage responses of a GoC pair to hyper- and depolarizing current pulses in the prejunctional cell [cell 2, filled with a quaternary lidocaine derivative (QX314) to block spiking]. **(H)** Spike frequency increase of all GoCs for networks with measured dendritic GJ distribution (left), with a homogeneous dendritic GJ distribution (middle), or with GJs restricted to the soma (right). **(I)** Spike frequency increase versus GJ conductance. **(J)** Proximal MF inputs strongly activate innervated GoC, but spread of synaptic charge to GJ-coupled GoCs is weak. Distal PF inputs weakly activate innervated GoC, but synaptic charge spreads efficiently to GJ-coupled GoCs, increasing their firing.

(fig. S13), and was strongest when the GoC population was sparsely activated by chemical excitatory synaptic inputs (fig. S14).

Our results show that the passive properties of GoC dendrites confer distance-dependent sub-linear chemical synaptic integration. This weakens the impact of distal excitatory inputs. However, the high density of dendritic GJs in the molecular layer enables PF synaptic charge to flow into the dendrites of neighboring GoCs. This GJ-mediated lateral excitation counteracts the effects of sub-linear dendritic behavior by enabling distal inputs to drive network activity more effectively. Dendritic GJs therefore counteract the problem of dendritic saturation (24) without the need to boost electrically remote synaptic input with active dendritic conductances (25). A key role of interneurons is to counteract and balance network excitation. The combination of passive dendrites and dendritic GJs facilitates this by enabling a larger fraction of interneurons to respond to localized patches of synaptic excitation. Our results reveal how GJs on inhibitory interneuron dendrites could contribute to spatial averaging, which has been proposed in the retina (26) and excitatory olfactory neurons in insects (27), and to the broad tuning of inhibitory interneurons in cortex (28). These mechanisms are also likely to contribute to gain control in the granule cell layer through PF-mediated feedback (29), and it seems likely that

interneurons in cortical and subcortical structures (7) use similar mechanisms. Our results suggest that interneurons do not operate as fully independent neuronal units but share charge during chemical synaptic excitation and thus exhibit features of a syncytium.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1215101/DC1
Materials and Methods
Figs. S1 to S15
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The Geometric Structure of the Brain Fiber Pathways

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The structure of the brain as a product of morphogenesis is difficult to reconcile with the observed complexity of cerebral connectivity. We therefore analyzed relationships of adjacency and crossing between cerebral fiber pathways in four nonhuman primate species and in humans by using diffusion magnetic resonance imaging. The cerebral fiber pathways formed a rectilinear three-dimensional grid continuous with the three principal axes of development. Cortico-cortical pathways formed parallel sheets of interwoven paths in the longitudinal and medio-lateral axes, in which major pathways were local condensations. Cross-species homology was strong and showed emergence of complex gyral connectivity by continuous elaboration of this grid structure. This architecture naturally supports functional spatio-temporal coherence, developmental path-finding, and incremental rewiring with correlated adaptation of structure and function in cerebral plasticity and evolution.

The organizing principles of cerebral connectivity remain unclear. In the brainstem and spinal cord, fiber pathways are organized as parallel families derived from the three principal axes of embryonic development: the rostro-caudal, the medio-lateral (or proximo-distal), and the dorso-ventral (1–6). In the forebrain of advanced species, however, corresponding patterns of connectivity have yet to be established. Many studies of evolution, development, and gene expression point to a geometric organization of cerebral fiber pathways similar to that of the brain-

stem (3–5, 7–9), and functional studies (10–13) also suggest that connectivity is geometrically organized. Several leading theories of cerebral function (14–17) propose geometric organization at multiple scales. However, high-resolution studies of cerebral connectivity with tract tracers have given only limited evidence of geometric organization (10–12, 18, 19).

A challenge in the investigation of cerebral structure and connectivity can be traced to the common occurrence of distinct pathways within the same small volumes of tissue, or “path cross-

ing.” Crossing is a pervasive feature of brain structure and may be essential for efficient connectivity (20, 21). Owing to crossing, the mapping of connectivity must untangle pathways from cellular to macroscopic scales simultaneously (22, 23). This was accomplished with tract tracers methods, which are considered a gold standard (18, 19). Tracer studies inject compounds into the live brain and allow them to disperse by means of axonal transport, marking individual axons over large distances. However, these can map only a small fraction of the pathways in any single brain and are not feasible in humans. Thus, the discovery and analysis of the structural relationships between pathways—and their context within cerebral connectivity—has remained challenging (18, 19, 24).

To address these limitations, methods have been developed to map the fiber pathways of the

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brain through use of diffusion magnetic resonance imaging (MRI). Diffusion MRI creates multi-dimensional contrast that is representative of the distribution of fiber orientations at each location in the tissue (21). Though of lower resolution than tract tracing, diffusion MRI is noninvasive, applicable to humans and synoptic, and able to map the connective anatomy of a single brain in its entirety, including spatial correlations between path-

ways. These correlations represent the mesoscale structure of connectivity, within the scope of which are the questions of whether cerebral pathways are discrete versus continuous and the detailed character of the spatial organization of connectivity.

To probe the spatial relations between the pathways of the brain, we analyzed path-adjacency and path-crossing in four nonhuman primate species and in humans. Diffusion spectrum MRI (DSI)

was acquired in whole-brain specimens *ex vivo* in rhesus, owl monkey, marmoset, and the prosimian galago, and *in vivo* in subjects (515 directions; pathways were computed with deterministic streamline integration) (21). To demonstrate pathways' structural relationships, we augmented interactive software to compute for any path the set of all paths with which it shares one or more voxels, termed its path neighborhood.

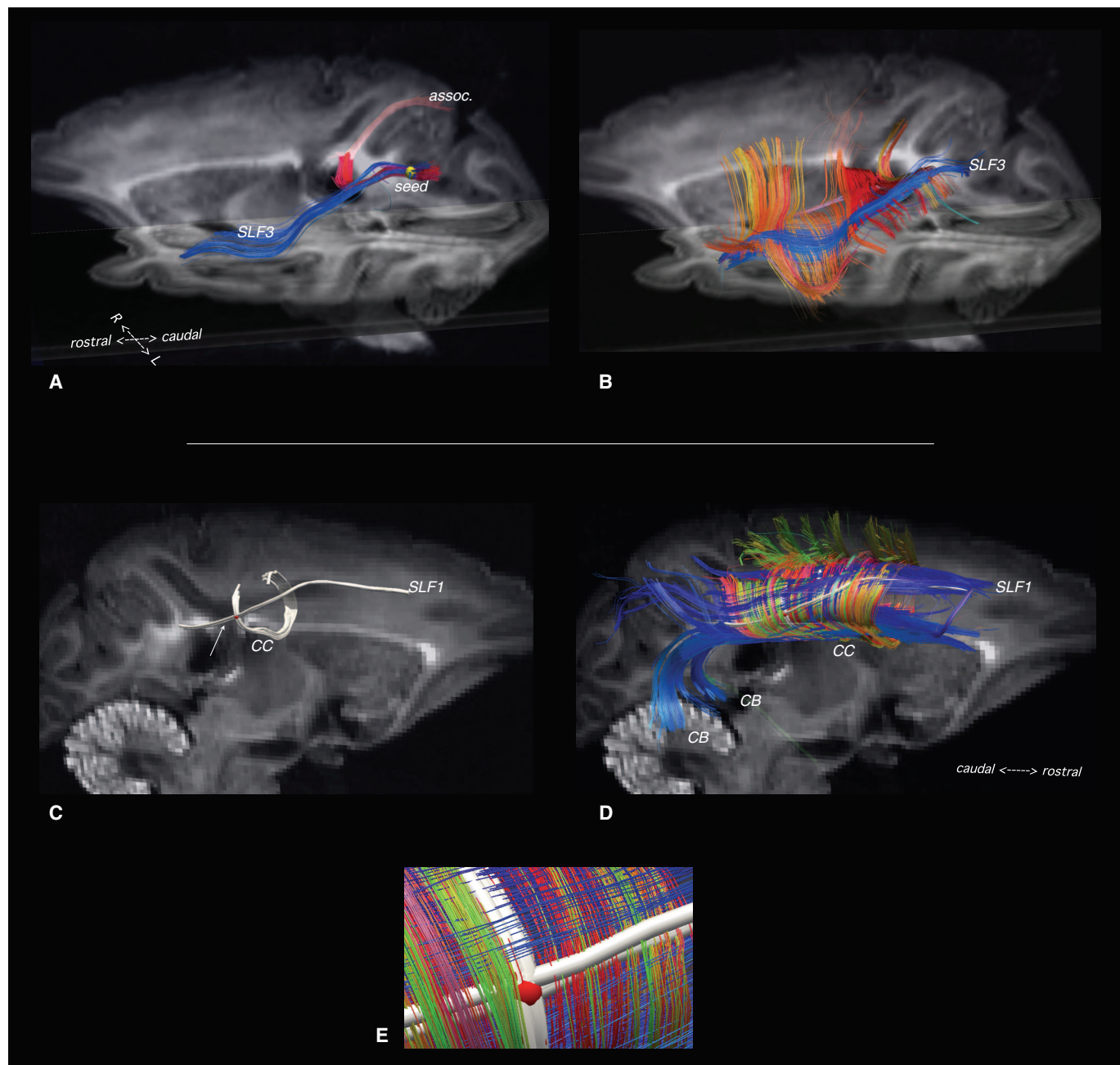


Fig. 1. Neighborhood structure of cerebral pathways. (A) MRI analog of tracer injection. A major association pathway of the rhesus forebrain SLF3 (blue) was identified by its termination in a selected region (yellow sphere in the parietal lobule). A co-terminus association pathway (red) is noted. These pathways appear as isolated structures. (B) The path neighborhood of SLF3 is identified: a curved sheet of parallel paths (orange) that cross SLF3 nearly orthogonally. (C to E) A path neighborhood in the rhesus frontal lobe (right lateral view). (C)

A region (red sphere, arrow) is selected in the white matter deep to the cingulate gyrus. The paths incident on this region (white) are near-orthogonal paths within the corpus callosum and SLF1. (D) The set of all paths incident on these paths in (C) forms a curved sheet of interwoven orthogonal pathways [(E) shows detail], including mutually parallel transverse paths of the corpus callosum (red to green) and longitudinal paths (blue) within SLF1 and the cingulum bundle (CB). No other path orientations were evident.

The character of a typical cerebral path neighborhood is illustrated in Fig. 1. In Fig. 1A, an association pathway—rhesus monkey superior longitudinal fasciculus-3 (SLF3)—is identified by its termination within the parietal lobe; this is the MRI analog of a tracer injection (25). So defined, this pathway appeared as an almost isolated structure. In Fig. 1B, the path neighborhood of SLF3 was identified and was astonishingly simple. It

entirely consists of a single curved two-dimensional (2D) sheet of paths, all mutually parallel, transversely oriented, and all crossing SLF3 at nearly right angles.

To investigate neighborhood structure independent of path identifications, we adopted the following procedure: select a small region, identify its incident paths, and compute the paths incident on these paths, their neighborhood. This was per-

formed in the rhesus frontal lobe, as illustrated in Fig. 1, C to E. The path neighborhood comprises two sets—transverse callosal paths and longitudinal paths of the cingulum and SLF1—that crossed like the warp and weft of a fabric as a near-orthogonal grid. Thus, these paths formed a single biaxial system. This pattern was typical of cerebral white matter.

The 3D structure of cerebral pathways was demonstrated through analysis of several path

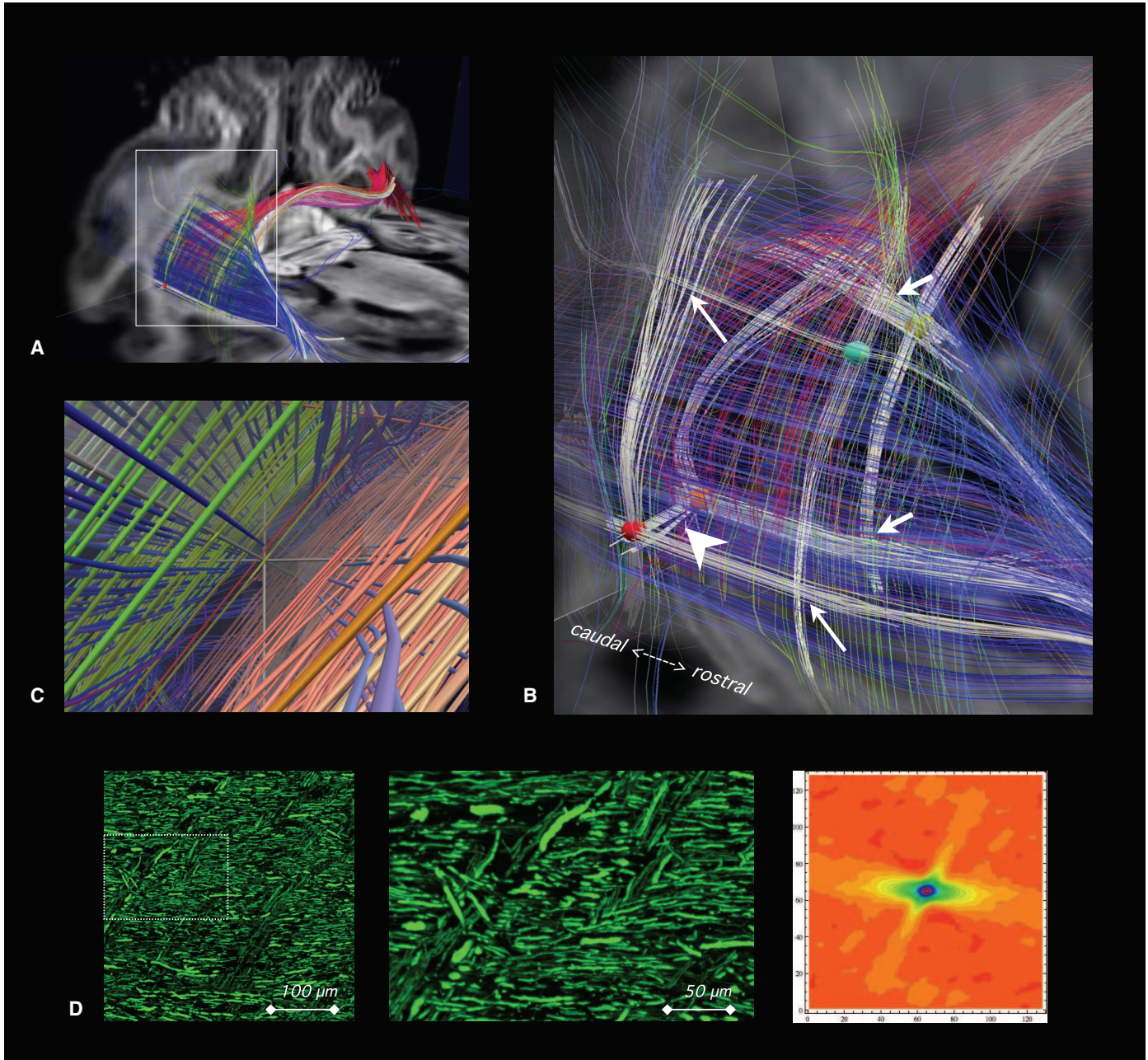


Fig. 2. Grid structure of cerebral pathways. The grid structure of the pathways of the sagittal stratum in the rhesus occipital lobe was demonstrated by means of neighborhood analysis. (A and B) Four seed regions were selected (spheres are indicated as superficial by red and green and as deep by yellow and orange), their paths identified (white), and neighborhoods computed (fronto-occipital fasciculus, blue; callosal paths, red and orange). The interior view along the axis of the structure (C) illustrates its character as an orthogonal grid. These neighborhoods comprised 2D sheets of closed quadrilaterals at

each depth [(B), arrows]. The paths within each sheet were orthogonal: of the longitudinal fronto-occipital fasciculus (FOF) (blue) or the transverse callum (red) and association system (green). Paths of the third mutually orthogonal direction (arrowhead), perpendicular to the local cortical surface, were noted. (D) Confocal microscopy of a sagittal slice oblique cut parallel to the lateral face of the sagittal stratum showed in-plane crossing of FOF (horizontal) and callosal paths (vertical oblique), and the 2D autocorrelation map of this microscopy, representative of the fiber orientations.

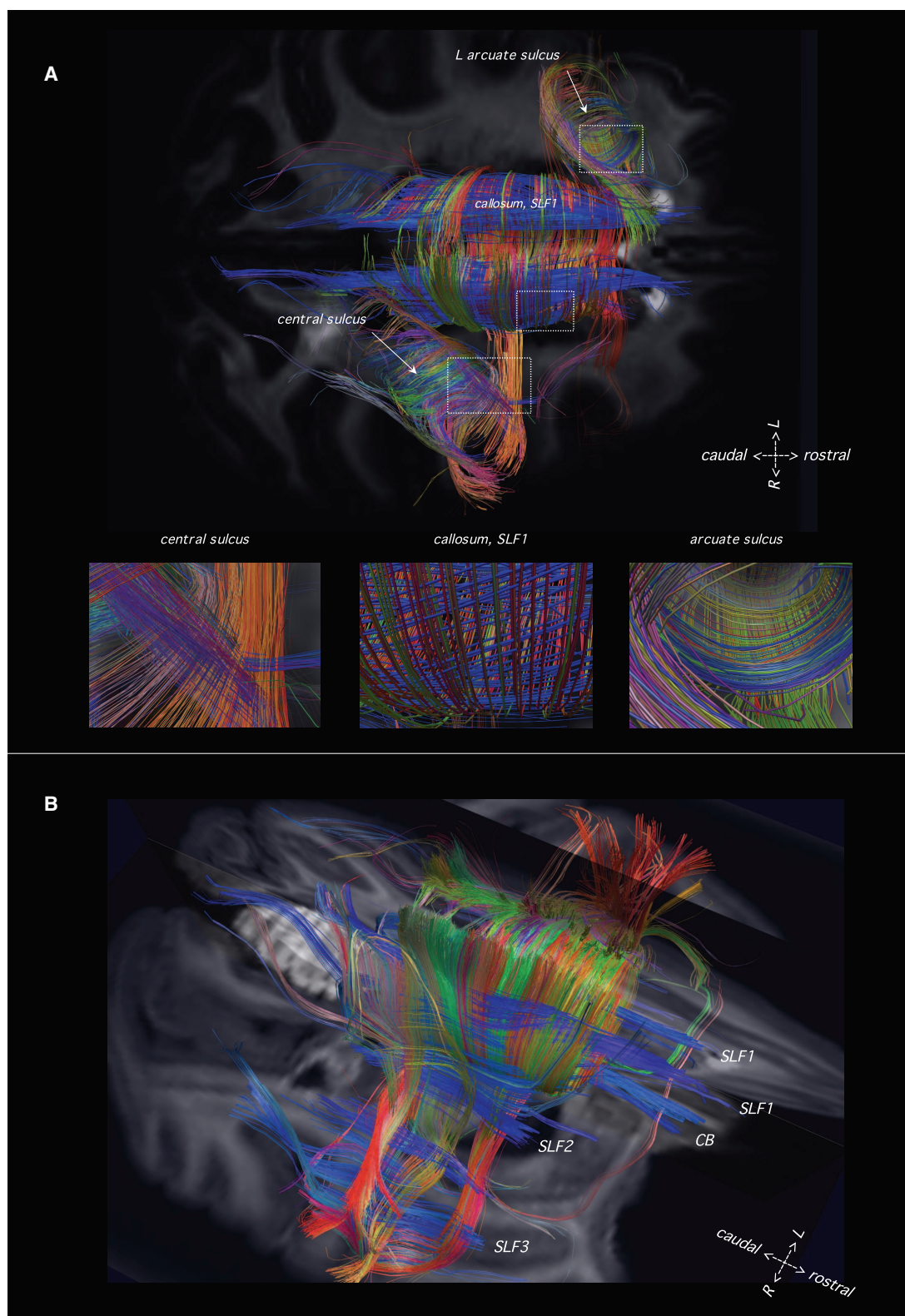
neighborhoods in a single region. Deep white matter of the occipital lobe—the sagittal stratum—of the rhesus monkey is shown in Fig. 2. Here, four seed regions were selected whose incident paths and their neighborhoods formed the edges and faces of a curved rectangular box, demonstrating

that parallel grid structure of pathways was not limited to particular 2D surfaces but extended throughout entire 3D volumes. Paths in the third nearly orthogonal axis were also seen and no diagonal paths observed. Similar 3D organization was demonstrated in the highly curved midline

system [supporting online material (SOM) text and fig. S1].

In all present studies, cerebral path crossings formed well-defined 2D sheets. For example, in Fig. 2B paths crossed (arrows) so as to form closed quadrilaterals that are elements of well-defined

Fig. 3. Continuous grid structure of rhesus frontal lobes. **(A)** The grid structure of subcortical pathways was continuous within and between path neighborhoods. Three neighborhoods were constructed of the left arcuate and right central sulci and the midline. (Insets) All cortico-cortical pathways were highly curved elements in a sheet of interwoven paths in two nearly perpendicular orientations. The grid structure was continuous within and between neighborhoods. Path orientation was aligned with gyral topography in the arcuate sulcus and but oblique in central sulcus, with spiral trajectories (violet). **(B)** Major pathways in deep white matter in the rhesus frontal lobe (from top left), including SLF 1-3, and the cingulum bundle (CB) (blue) were components of a single path-grid. Their intersecting pathways (green, orange, and red) are transverse, parallel at all scales, and cross orthogonally.



2D surfaces. Geometrically, this configuration is highly exceptional (26, 27). Just as it is exceedingly unlikely that several points fall on a straight line, it is similarly improbable that two families of curves in 3D have any 2D surfaces in common. This sheet structure was found throughout cerebral white matter and in all species, orientations, and curvatures. Moreover, no brain pathways were observed without sheet structure. Further, because the processes of diffusion encoding, recon-

struction, and tractography are purely local, limited to single or to adjacent voxels, whereas the spatial correlations entailed in this pattern were long-range and nonlinear, this structure could not be attributed to technical artifacts related to the imaging of diffusion (SOM text and fig. S2). A histological counterpart of the crossing structure of fiber pathways was observed with confocal microscopy: a tissue section through the rhesus monkey sagittal stratum cut parallel to the sheets

of Fig. 2, A to C, that showed axons interweaving and crossing in two axes, which was confirmed by the orientation distribution of their spatial autocorrelation (28).

Grid structure of cerebral pathways was pervasive, coherent, and continuous with the three principal axes of development. In each region in Fig. 3A, continuous grid structure is demonstrated across several regions in the frontal lobes in rhesus monkey, including left arcuate sulcus,

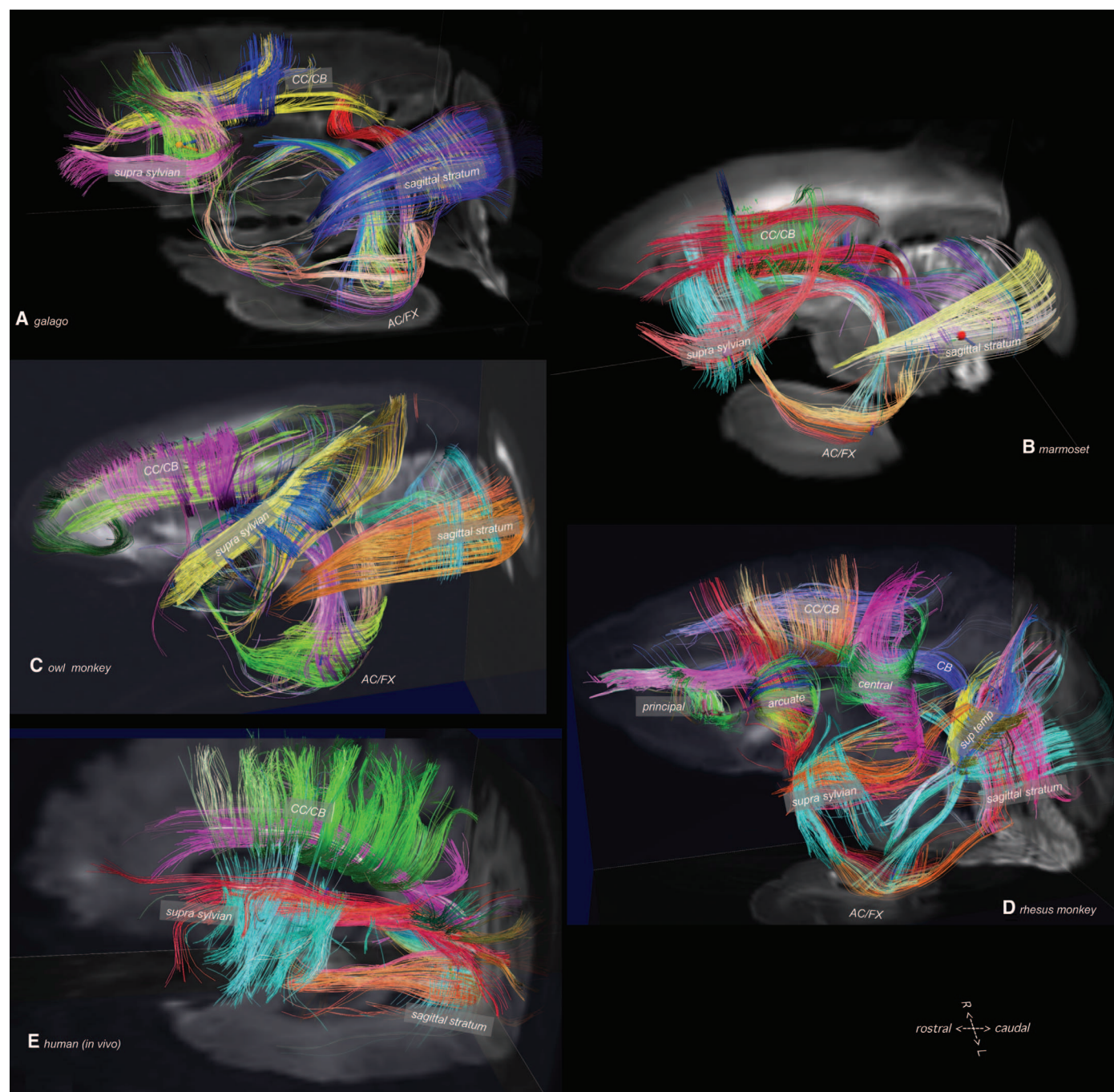


Fig. 4. Homologous cerebral grid structure in (A) galago, (B) marmoset, (C) owl monkey, (D) rhesus monkey, and (E) human, left lateral views. Homologous grid structures including those of the corpus callosum/cingulum bundle (CC/CB); sagittal stratum and supra-Sylvian region were identified in all species, and that

of the anterior commissure and fornix (AC/FX) was resolved in all ex vivo studies [(A) to (D)]. In the rhesus monkey, grid structure is shown in gyri and sulci, including the principal, arcuate, and central sulci and the superior temporal gyrus, continuous as grids with those of the adjacent deep white matter.

midline callosal region, and right central sulcus. The grid orientations in central sulcus were not parallel to gyral topography but were oblique, following spiral trajectories. This contrasted with the temporal lobes, where path and gyral orientations were closely aligned (SOM text and fig. S3A).

In rhesus deep white matter, the major frontal association pathways were parallel elements of a single grid (Fig. 3B, SOM text, and fig. S4). These pathways were not highly distinct but continuous with their neighbors via sparse interposed parallel paths. This was consistent in analysis at multiple depths (SOM text and fig. S4). Thus, major longitudinal pathways of the frontal lobe as well as longitudinal U-fiber pathways could be considered local condensations of a single system that spans the frontal lobe. The grid structure of pathways of the cerebral mantle was continuous with those of the limbic system and basal ganglia (SOM text and fig. S3B).

To investigate how pathways may change course, we analyzed a known turn in a major cerebral pathway identified in the tracer studies of Schmahmann and Pandya in rhesus monkey (17). Tracer and DSI showed bifurcation of a frontal projection pathway into transverse and dorso-ventral components. In lieu of diagonals, pathways were found to bifurcate and turn between axes (SOM text and fig. S5).

To investigate grid continuity in the cerebral hemisphere, widely separated path neighborhoods were constructed in owl monkey. Grid structure was maintained at all scales, from the single voxel, to the lobe, to the hemisphere (SOM text and fig. S6). Continuity of grid structure between superficial gyral and deep cerebral pathways was demonstrated through analysis of neighborhoods at sequential depths (SOM text and fig. S4). Grid structure in all three orthogonal axes was observed in the centrum semiovale, including the longitudinal and transverse paths and dorso-ventral projection paths. This was observed in the rhesus and in the human, in vivo and ex vivo (SOM text and fig. S7). Further validation of grid structure of cerebral pathways was obtained with an alternative MRI contrast mechanism of circular diffusion contrast (SOM text and fig. S8) (29), and the recognized triaxial structure of pathways of the brainstem was shown with present methods (SOM text and fig. S9).

Strong homology of deep cerebral grid structure was found across all species studied (Fig. 4). These included the grid systems of the callosum, sagittal stratum, and supra-Sylvian pathways, as well as the crossing of the fornix and anterior commissure in all species studied ex vivo at high resolution. In the rhesus monkey, central and sub-cortical grid structures (Fig. 4D), including those of the major frontal sulci (principal, arcuate, central), fit together continuously like a jigsaw puzzle (SOM text and figs. S3A, S4, and S6, owl monkey). Thus, we hypothesize that the complex connectivity of the cerebral mantle represents a continuous elaboration of the simpler core.

We have found that the fiber pathways of the forebrain are organized as a highly curved 3D grid derived from the principal axes of development. This structure has a natural interpretation. By the Frobenius theorem, any three families of curves in 3D mutually cross in sheets if and only if they represent the gradients of three corresponding scalar functions (26, 27). Accordingly, we hypothesize that the pathways of the brain follow a base-plan established by the three chemotactic gradients of early embryogenesis (30). Thus, the pathways of the mature brain presents an image of these three primordial gradients, plastically deformed by development. This could be tested by obtaining diffusion MRI during cerebral embryogenesis and assessing the derivation of pathways from the primordial directions. Grid structure should restrict and simplify axonal path-finding compared with models that allow less constrained and less correlated connectivity within and between cerebral areas. If grid structure guides connectivity similar to the lane markers in a highway, then navigation would be reduced from a general 3D problem to a far simpler question of when to exit. In the brain, fibers growing in any axis would have a choice at each moment of just the four orthogonal directions perpendicular to their course. Grid structure would increase the efficacy of path orientation as a mechanism of axonal path-finding (31, 32). Simultaneously, this structure supports incremental modification of connectivity by geometric modification within broad continuous families of parallel paths. Thus, the grid organization of cerebral pathways may represent a "default connectivity," on which adaptation of structure and function can both occur incrementally in evolution and development, plasticity, and function.

A grid organization of cerebral pathways has been suggested in several contexts. Katz *et al.* (8) hypothesized that a large-scale substrate of grid organization is applicable to forebrain development. Checkerboard organization of cortical Brodmann fields has been noted in visual areas of the temporal lobe (1) and frontal motor areas (3) in monkeys. In humans, Badre and D'Esposito (13) have suggested hierarchic organization of the frontal cortex along a rostro-caudal axis.

The correlation between grid and topographic orientations observed in the rhesus temporal lobe supports Van Essen's hypothesis relating cerebral folding to fiber tension (33); however, the variable relation of fiber structure and cortical folding observed in the frontal lobe merits further investigation. More recently, Clochoux *et al.* have derived from its patterns of folding a spherical coordinate system for the longitude and latitude of the human cerebral cortex (34). This coordinate system seems generally congruent with that implied by the present grid structure of pathways, and investigation of their relationship may shed light on the linkage in structure and development between cerebral gray matter and white matter.

Greater differentiation between pathways was observed in the more complex rhesus brain than in

simpler species, suggesting that the evolutionary emergence of discrete pathways parallels the increasing cerebral complexity in the primate lineage. Functionally, pervasive cerebral organization with parallel paths and similar lengths would naturally support neural coding via spatial and temporal coherence (16).

The grid structure of cerebral pathways has implications for brain mapping: It suggests a simplifying framework and natural coordinate system (35) for the description of brain structure, its pathways, and connectivity; simplifies and constrains models of cerebral white matter; and indicates that topographic organization is characteristic of cerebral connectivity and not limited to a few major pathways. Of concern, present findings suggest that existing MRI tractography may underestimate sharp turning (36). It also provides a means to validate MRI tractography through consistency with grid structure.

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Hierarchical Genetic Organization of Human Cortical Surface Area

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Surface area of the cerebral cortex is a highly heritable trait, yet little is known about genetic influences on regional cortical differentiation in humans. Using a data-driven, fuzzy clustering technique with magnetic resonance imaging data from 406 twins, we parceled cortical surface area into genetic subdivisions, creating a human brain atlas based solely on genetically informative data. Boundaries of the genetic divisions corresponded largely to meaningful structural and functional regions; however, the divisions represented previously undescribed phenotypes different from conventional (non-genetically based) parcellation systems. The genetic organization of cortical area was hierarchical, modular, and predominantly bilaterally symmetric across hemispheres. We also found that the results were consistent with human-specific regions being subdivisions of previously described, genetically based lobar regionalization patterns.

As early as the 1950s, Bergquist and Kallen postulated that the entire embryonic brain is divisible into an anteroposterior series of segmented neuromeres, each forming a complete ring around the brain's longitudinal axis (1). Almost 40 years later, experimental data showed that many gene expression domains respect segment boundaries in the embryonic vertebrate hindbrain, suggesting a role of genetic

control in regional differentiation (2, 3). This important finding prompted a search for similar genetic regulatory organization in other regions of the developing vertebrate brain (4). In particular, in the past decade the cerebral cortex has received substantial attention. Studies have shown, for example, that several signaling molecules and transcription factors are involved in establishing boundaries between mouse cortical regions (5, 6). Animal data demonstrate that the regional or positional identity of cortical regions is defined by the combinatorial expression pattern of various genes controlling for regional differentiation, each of which is expressed in a graded and restricted pattern with distinct spatiotemporal characteristics (7). Little is known, however, about the genetic patterning underlying the human cortex. In our previous work (8), we showed that genetic patterning underlying the anteroposterior gradient and four basic cortical divisions of cortical surface area demonstrated in mouse models (7) also existed in the human cortex. Furthermore, region-specific cortical areal expansion in humans has been linked to specific genetic polymorphisms (9, 10). We sought to go beyond the fundamental commonalities that humans share with other species and to investigate the genetic patterning specific to the human cortex with its

1000-fold increase in surface area relative to the mouse brain (11). In effect, we sought to develop a brain atlas of human cortical surface area that was based entirely on genetic correlations, rather than a priori structural or functional information.

To delineate the genetic patterning of the cortical area, we measured relative surface areal expansion using cortical surface reconstruction and spherical atlas mapping developed by Dale and colleagues (12–14). We divided the area measured at each location by the total surface area in order to account for global effects. Using the twin design, which compares monozygotic and dizygotic twins, we then estimated genetic correlations between different points on the cortical surface. These genetic correlations represent shared genetic influences on relative areal expansion between cortical regions (15). Details of these methods have been previously described (8, 16). After computing pairwise genetic correlations, we used an unsupervised pattern recognition method—fuzzy cluster analysis (17)—to demarcate the genetic topography of cortical surface area based on the genetic correlations of relative surface area measures. To determine the appropriate number of clusters, we computed the widely used silhouette coefficient.

On the basis of the peak of the silhouette coefficients (fig. S1), we identified 12 natural clusters. These clusters correspond closely to meaningful structural and functional regions (Fig. 1), even though the registration procedure did not rely on prespecified anatomical landmarks; rather, it makes use of the continuous pattern of surface curvature (13). In describing the subdivisions, we use conventional labels, but these only approximate the observed clusters. Subdivisions of the frontal cortex include the motor-premotor, dorsolateral prefrontal cortex extending to the anterior and superior parts, dorsomedial frontal, and orbitofrontal (Fig. 1, clusters 1 to 4). Another cluster is found between the frontal and parietal cortices, extending from pars opercularis to the subcentral region, including the inferior pre- and post-central gyri (Fig. 1, cluster 5). The temporal cortex includes the superior temporal, posterolateral temporal cortex extending to temporal and parietal junction, and anteromedial temporal cortex (Fig. 1, clusters 6 to 8). The parietal

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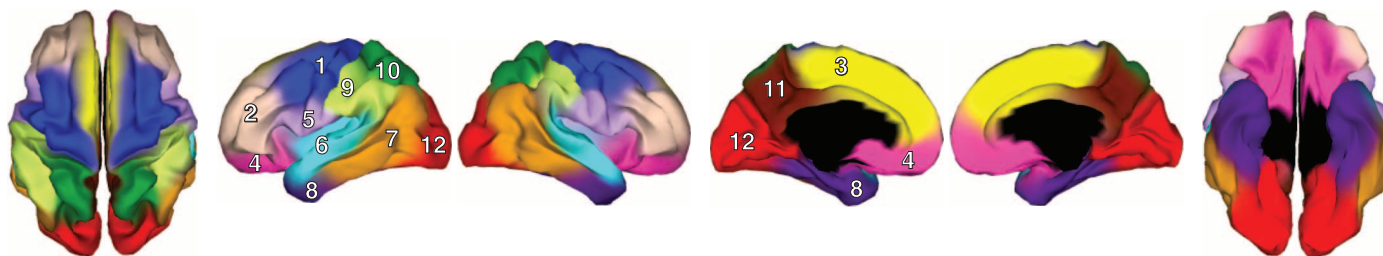


Fig. 1. Genetic clustering map for 12-cluster solution. 1, motor-premotor cortex; 2, dorsolateral prefrontal cortex; 3, dorsomedial frontal cortex; 4, orbitofrontal cortex; 5, pars opercularis and subcentral region; 6, superior temporal cortex; 7, posterolateral temporal cortex; 8, anteromedial temporal

cortex; 9, inferior parietal cortex; 10, superior parietal cortex; 11, precuneus; and 12, occipital cortex. Views shown from left to right are, respectively, superior, left hemisphere lateral, right hemisphere lateral, left hemisphere medial, right hemisphere medial, and inferior.

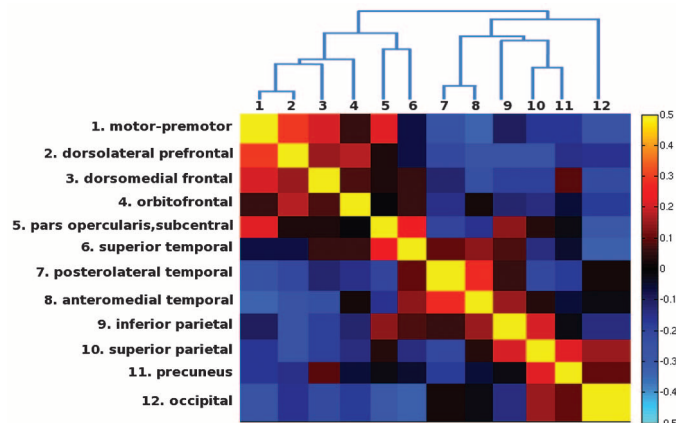
cortex includes the inferior parietal cortex, superior parietal cortex, and precuneus (Fig. 1, clusters 9 to 11). The occipital cortex constitutes a single cluster (Fig. 1, cluster 12). Some anatomical boundaries of these clusters map onto traditionally parcellated regions, such as cytoarchitectural areas or gyrus patterns; however, others do not follow classically defined boundaries (such as Brodmann areas). For example, there is no natural sulcal-gyral boundary between our dorso-medial and orbitofrontal clusters, but they still correspond reasonably well to the division between Brodmann areas 10 and 11. Conversely, the well-defined cytoarchitectural differentiation between Brodmann areas 17 and 18 is not manifest as separate genetically based clusters in our analyses.

The genetically based clusters presented a spatially contiguous pattern within hemispheres. However, the cluster algorithm placed no constraint against noncontiguous clusters. Indeed, all 12 clusters were noncontiguous clusters bilaterally located in the homologous regions between hemispheres. There were some indications of surface-area asymmetry around perisylvian regions (8), but the patterns of the left and right hemispheres were almost mirror images of one another. Because the clustering was conducted on both hemispheres simultaneously with no constraint for hemispheric symmetry, the results clearly indicate a predominantly bilateral symmetric and within-hemisphere modular pattern.

In order to obtain reliable estimates of the genetic correlations, we applied spatial smoothing, which limited our ability to address the fine spatial structure of the genetic patterning (16). We focused on the large-scale, primary structure of genetic patterning. Other techniques, such as gene expression analysis of brain tissue, could reveal finer-scale genetic patterning that may show more asymmetrical features or more subdivisions (18, 19) than can our magnetic resonance imaging (MRI)-based approach.

After identifying the boundaries of the genetically based parcellation, we next sought to examine the genetic relations between the 12 clusters; in particular, we searched for underlying organizational principles among these genetic subdivisions. We calculated the genetic similarity matrix to determine the genetic relatedness be-

Fig. 2. Genetic similarity matrix and dendrogram. The color scale represents the weighted mean genetic correlations within and between clusters. Negative genetic correlations indicate that the genes that cause areal expansion in anterior regions also cause relative areal contraction in posterior regions and vice versa (8).



tween clusters (Fig. 2). We found that genetic correlations are higher between clusters within the same lobe than between clusters in different lobes. Also included in Fig. 2 is a dendrogram derived from hierarchical clustering that summarizes the genetic relations between clusters. The dendrogram depicts a hierarchical structure of genetic patterning. The most distinct genetic partitions located at the highest level of the hierarchy correspond to the basic anteroposterior division between motor and sensory cortices; below that are the functionally specialized subdivisions generally nested within lobes. Similarly, a clear basic frontal/nonfrontal division and lobar-like clusters have been revealed by hierarchical clustering derived from transcriptome analyses of the fetal human brain (20, 21). One exception to these general patterns is that clusters belonging to the perisylvian region have relatively high correlations with one another, even though they are in different lobes. Cross-lobe clustering in these regions is consistent with a human-specific subdivision specialized for language.

We also examined the progression of cluster solutions, from 2 to 12 clusters, using fuzzy clustering (fig. S2). If the structure of the data are hierarchical, then successive clusters will tend to be subdivisions of previous clusters (22). In contrast to hierarchical clustering, our approach imposed no constraint for hierarchical organization; each level of the fuzzy clustering analysis was performed independently. Yet, the sequentially unfolding pattern revealed that the emerging

clusters tended to respect the boundaries of preceding clusters and appeared to be nested subdivisions. The convergence of results of this analysis and the dendrogram method thus provide further evidence for a hierarchical structure of genetic patterning that is intrinsic to the data.

The organization of genetic patterning is consistent with a ubiquitous pattern in the development of biological forms—increasing differentiation along with what appeared to be increasing hierarchical integration, as reflected by functionally specialized subdivisions (23). We previously showed that the four-cluster solution revealed fundamental genetic divisions comprising primary functional regions largely corresponding to the lobar divisions in all mammalian species (8). Our current results demonstrate further differentiation of each of the lobes into several nested subdivisions that correspond specifically to human functional specialization, such as the lateral or granular prefrontal cortex, and regions around Broca's area and the subcentral region associated with vocalization essential for human language (24). Our results suggest that human specialization regions are not genetically more distinct than primary functional lobar regions. However, small genetic differences resulting in functional importance have become increasingly recognized (11, 25). These findings support the notion that the human cortex is built on the foundation of the primary functional divisions, which are shared among mammals (7). Without any incorporation of prior anatomical knowledge, this statistically constructed

hierarchy demonstrated a biologically sensible organizational structure of the human brain.

We described a previously unidentified parcellation system for the human cortex that reflects shared genetic influences on cortical areal expansion. This system constitutes the first human brain atlas based solely on genetically informative data, which may provide presently undescribed phenotypes that will have greater statistical power for genome-wide genetic association studies in comparison with traditional cortical parcellations. We found evidence for a hierarchical, modular, and bilaterally symmetric genetic architecture. Genetically based lobar regions have been demonstrated across mammalian species (7, 8), and our results are consistent with genetically based regions of human specialization being increasingly differentiated subdivisions of these lobar regions. Our findings may thus be useful for translating results from model organisms into functional and clinical insights about human specializations, so as to understand both order and disorder in the human brain.

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Supporting Online Material

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Materials and Methods

Figs. S1 to S9

Table S1

References

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Ecological Context Influences Epidemic Size and Parasite-Driven Evolution

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The occurrence and magnitude of disease outbreaks can strongly influence host evolution. In particular, when hosts face a resistance-fecundity trade-off, they might evolve increased resistance to infection during larger epidemics but increased susceptibility during smaller ones. We tested this theoretical prediction by using a zooplankton-yeast host-parasite system in which ecological factors determine epidemic size. Lakes with high productivity and low predation pressure had large yeast epidemics; during these outbreaks, hosts became more resistant to infection. However, with low productivity and high predation, epidemics remained small and hosts evolved increased susceptibility. Thus, by modulating disease outbreaks, ecological context (productivity and predation) shaped host evolution during epidemics. Consequently, anthropogenic alteration of productivity and predation might strongly influence both ecological and evolutionary outcomes of disease.

Parasites can impose strong evolutionary pressure on their hosts during epidemics (1, 2). Parasites often virulently depress survival and/or birth rate of their hosts. As a result, if epidemics become large enough, host populations might evolve resistance to infection because of parasite-mediated directional selection (1). Alternatively, if the susceptibility of a host genotype depends on the parasite genotype to which it is

exposed, negative frequency-dependent selection can drive cycling of host genotypes through time [that is, "Red Queen dynamics" (3, 4)]. These two ideas about host (co-)evolution during epidemics, evolution of increased resistance and the Red Queen hypothesis, dominate research on evolutionary epidemiology (1). However, theory reveals other possibilities, including the evolution of higher susceptibility to infection (1, 5–8). Why would hosts evolve greater susceptibility to their virulent parasites during epidemics? When would host populations evolve this way in nature?

The answers to these questions involve trade-offs and ecologically driven variation in disease prevalence. Resistance to virulent parasites can trade off with reproduction; some genotypes have

higher fecundity but lower disease resistance, whereas others are less fecund but more resistant. The fittest strategy, then, depends on the net balance between resisting infection and enhancing fecundity. That balance, in turn, depends on ecologically determined disease prevalence. Environments with high resources for hosts (higher productivity) and lower mortality (lower predation) on hosts should fuel large epidemics (9–12). In these systems, theory predicts that hosts should evolve increased resistance to disease, even though resistant genotypes have lower fecundity. However, when low productivity and/or higher predation constrain epidemic size, populations should become more susceptible because more susceptible genotypes are more fecund.

We test these predictions in a host-parasite system that exhibits the requisite trade-offs and ecologically driven variation in epidemics. Clonal genotypes of the zooplankton grazer *Daphnia dentifera* face a trade-off between fecundity and resistance to infection by a virulent yeast parasite [*Metschnikowia bicuspidata* (13)]. Mechanistically, the resistance-fecundity trade-off is driven by variation in feeding rate: Slow feeders consume fewer free-living propagules (spores) of the yeast (conferring higher resistance) but assimilate energy less quickly (yielding fewer offspring). Neither host-parasite genotype specificity nor Red Queen dynamics appear in this system; host resistance does not depend on the parasite genotype to which it is exposed (14). This parasite reduces fecundity and survival (15). Epidemics erupt commonly in *Daphnia* populations, with maximal infection prevalence sometimes exceeding 60% (16, 17).

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Large epidemics depress host density (16), and host populations evolve rapidly during epidemics (14, 18, 19). Epidemics can grow larger in lakes with high nutrient concentrations [an index of productivity (20)]. In contrast, vertebrate predation depresses yeast epidemics, particularly be-

cause fishes selectively cull infected *Daphnia* (15, 21). Overall, we hypothesized that lakes with higher productivity and/or lower fish predation should have larger epidemics that should select for greater disease resistance in hosts. Conversely, lakes with lower productivity and/or higher predation should have smaller epidemics and hosts might evolve increased susceptibility.

To quantify epidemic size, we monitored epidemics and indices of productivity and predation in weekly sampling visits to seven lakes located in Greene and Sullivan Counties, Indiana. We estimated infection prevalence visually on live hosts by using established survey methods (15, 16, 21). To calculate epidemic size, we integrated the area under the time series of infection prevalence for each lake. This measure correlates strongly with maximum infection prevalence ($r = 0.89$, $P = 0.008$). We also estimated two indices of productivity, total phosphorus (P) and total nitrogen (N), by using standard methods [colorimetric assays and ultraviolet spectrophotometry, respectively (22)]. On the basis of ratios of nitrogen:phosphorus measured, productivity in these lakes is likely colimited or even nitrogen-limited (23, 24).

Mean length of uninfected adult hosts provided an index of predation pressure; smaller mean length indicates greater fish predation (25, 26).

To characterize host evolution during epidemics, we conducted infection assays for each lake population. To establish isofemale lines, we randomly isolated individual hosts at two time points: in late July before epidemics began (pre-epidemic) and in mid-November as epidemics waned but before hosts produced sexual females (postepidemic). We used those lines (9 to 21 per lake per period, mean of 15.4) to estimate mean infection risk of host populations (13, 14, 18) [also supporting online material (SOM)]. Here, infection risk reflects the product of spore uptake and infectivity of the spores once consumed (i.e., per-spore susceptibility). We refer to higher infection risk as “higher susceptibility” and lower infection risk as “higher resistance.” All assays were performed by using a single isolate of the yeast, because *Metschnikowia* collected from different lakes and years do not vary in relevant epidemiological parameters (14). We then analyzed infection data for each lake with a logistic regression model built with binomial errors and a logit

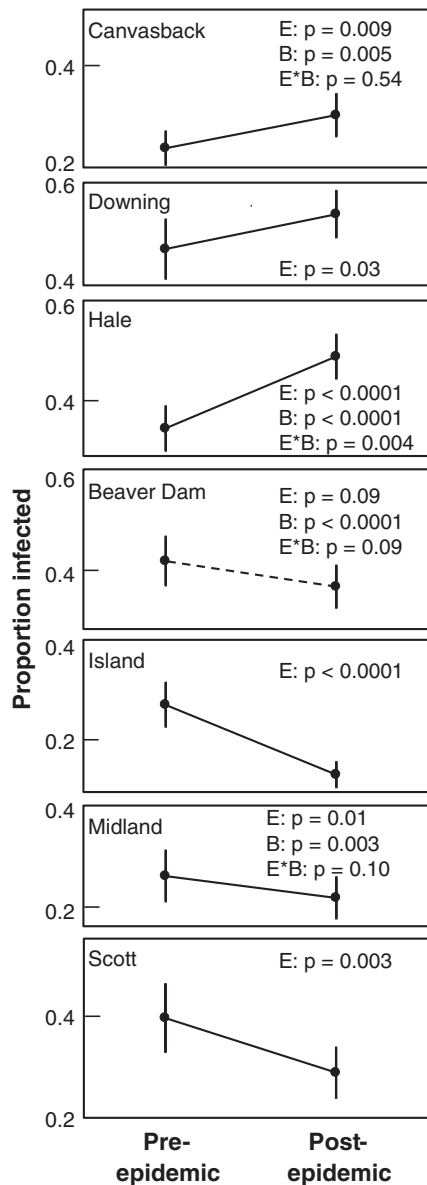


Fig. 1. Change in infection risk of seven populations of a zooplankton host (*D. dentifera*) during epidemics of a virulent yeast parasite (*M. bicuspidata*). Proportion infected from pre- and postepidemic lines are shown (means of clonal lines $\pm$ SE). E indicates the comparison of the pre- and postepidemic time periods; when applicable, B denotes time blocking, and E*B represents their interaction. The populations in Canvasback, Downing, and Hale Lakes became significantly more susceptible during their epidemics, whereas those in Island, Midland, and Scott Lakes became significantly more resistant. There was no significant change in Beaver Dam Lake (hence the dashed line connecting the two means).

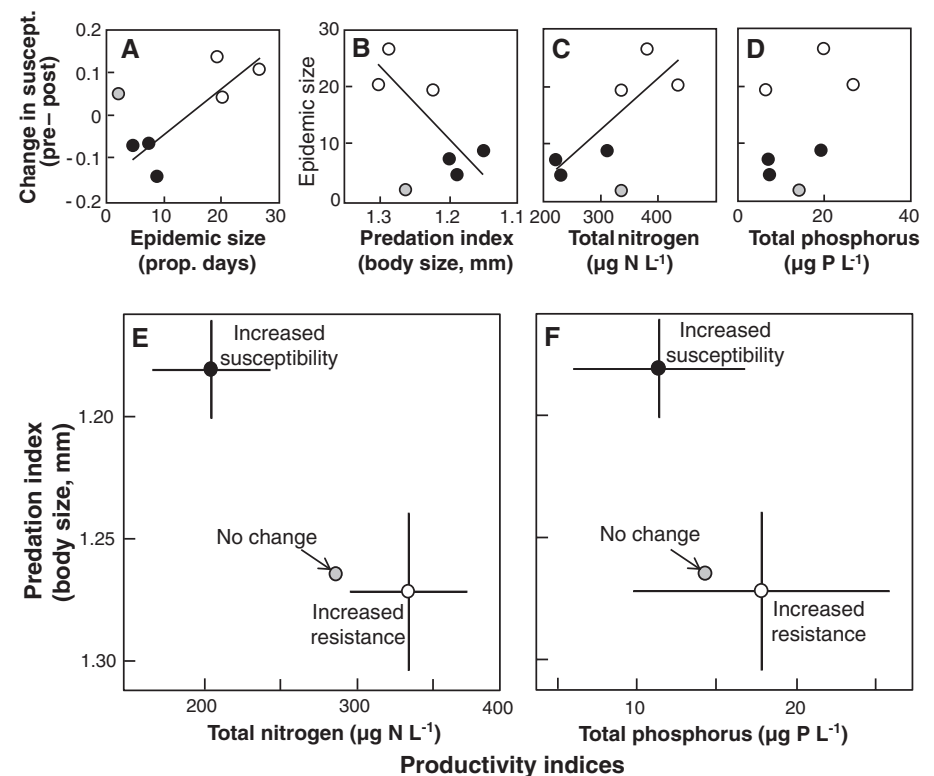


Fig. 2. Relationships between epidemic size, predation, productivity, and evolutionary outcomes. Top panels show links with epidemic size (quantified as the area under the infection prevalence curve through time). (A) Epidemic size versus change in mean susceptibility (mean proportion infected of postepidemic genotypes subtracted from mean of preepidemic genotypes). (B to D) Epidemic size is on the y axis, plotted versus (B) predation intensity (where smaller body size indicates higher predation) or productivity as indexed by (C) total nitrogen and (D) total phosphorus. Open symbols denote lake populations that evolved increased resistance (as shown in Fig. 1), black symbols indicate increased susceptibility, and gray symbols indicate no significant evolutionary change. (E and F) Evolutionary outcomes mapped onto predation-productivity space. Note that the y axis scales with increasing predation intensity, so small body size (high predation) is at the top. Points in (E) and (F) are lake means (± 1 SE).

link function (Proc Genmod, SAS 9.1, SAS Institute Incorporated, Cary, North Carolina). When the infection assays were run over two time blocks, the model also included a block effect and a time-by-block interaction.

The infection assays showed a significant evolutionary response of hosts to epidemics in six of seven lake populations. In three lakes (Island, Midland, and Scott Lakes), host populations became significantly more resistant during epidemics (Fig. 1). However, in three other populations (Canvasback, Downing, and Hale Lakes), hosts became significantly more susceptible to infection. The hosts in the seventh lake, Beaver Dam, did not show a significant change in susceptibility but trended toward increased resistance.

As anticipated by theory (SOM), these evolutionary trajectories correlated with ecologically driven variation in epidemic size. Among the six lake populations showing a significant evolutionary response, change in mean susceptibility correlated strongly with epidemic size (Pearson correlation: $r = 0.86$, $P = 0.030$, $n = 6$; Fig. 2A). Further, in those six lakes, epidemics were larger at lower predation intensity (larger size of hosts; Pearson correlation: $r = 0.86$, $P = 0.029$, $n = 6$; Fig. 2B) and where total nitrogen was higher (Pearson correlation: $r = 0.83$, $P = 0.040$, $n = 6$; Fig. 2C); the trend was similarly directed, but not significant, for total phosphorus (Pearson correlation: $r = 0.50$, $P = 0.3$, $n = 6$; Fig. 2D). Overall, hosts became more susceptible to the yeast in lower productivity lakes with higher vertebrate predation but evolved toward decreased susceptibility in more productive lakes with lower vertebrate predation (Fig. 2, E and F; t tests for differences between two groups; results for body size, $t_4 = 3.19$ and $P = 0.033$; nitrogen, $t_4 = 3.18$ and $P = 0.034$; phosphorus, $t_4 = 0.88$ and $P = 0.43$). Thus, ecological gradients, through their effects on epidemic size, influenced evolutionary outcomes of hosts during outbreaks of a virulent parasite. These qualitative predictions also arose from a general, trait-based epidemiological model built for similar epidemiology and parameterized for our particular system (SOM).

These results show that hosts can evolve enhanced susceptibility to their virulent parasites during epidemics [also see (27) for a similar but unreplicated occurrence]. A combination of observations, experiments, and modeling all suggest causation for this initially counterintuitive finding. When ecological factors promote large epidemics, hosts should evolve to become more resistant to infection. However, resistance-fecundity trade-offs can prompt host populations to evolve increased susceptibility when ecology constrains epidemic size. Overall, we demonstrated that ecological context influences epidemic size, which, in turn, determines evolutionary responses of hosts to epidemics. This suggests that alteration of predation pressure on hosts and productivity of ecosystems may influence the ecology and evolution of host-parasite interactions.

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Supporting Online Material

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Materials and Methods

Fig. S1

Tables S1 and S2

References (28–32)

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Rapamycin-Induced Insulin Resistance Is Mediated by mTORC2 Loss and Uncoupled from Longevity

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Rapamycin, an inhibitor of mechanistic target of rapamycin complex 1 (mTORC1), extends the life spans of yeast, flies, and mice. Calorie restriction, which increases life span and insulin sensitivity, is proposed to function by inhibition of mTORC1, yet paradoxically, chronic administration of rapamycin substantially impairs glucose tolerance and insulin action. We demonstrate that rapamycin disrupted a second mTOR complex, mTORC2, in vivo and that mTORC2 was required for the insulin-mediated suppression of hepatic gluconeogenesis. Further, decreased mTORC1 signaling was sufficient to extend life span independently from changes in glucose homeostasis, as female mice heterozygous for both mTOR and mTORC2 exhibited decreased mTORC1 activity and extended life span but had normal glucose tolerance and insulin sensitivity. Thus, mTORC2 disruption is an important mediator of the effects of rapamycin in vivo.

Age-related diseases—including cancer, neurodegenerative disorders, cardiovascular disease, type II diabetes, and many others—are the major contributors to morbidity and mortality in Western society. The high frequency of these diseases in the elderly limits the benefit that can be obtained by targeting them individually (1). However, targeting the aging process directly may offer a way to delay the incidence of many age-related diseases simulta-

neously. To date, the only molecule that appears to influence the intrinsic rate of aging in mammals, as evidenced by a robust extension of maximum life span, is rapamycin, an inhibitor of mechanistic (previously referred to as mammalian) target of rapamycin complex 1 (mTORC1) (2, 3).

mTOR is a kinase that integrates inputs from many nutrients and growth factors. mTOR is found in two distinct protein complexes: mTORC1, which regulates numerous cellular processes related to

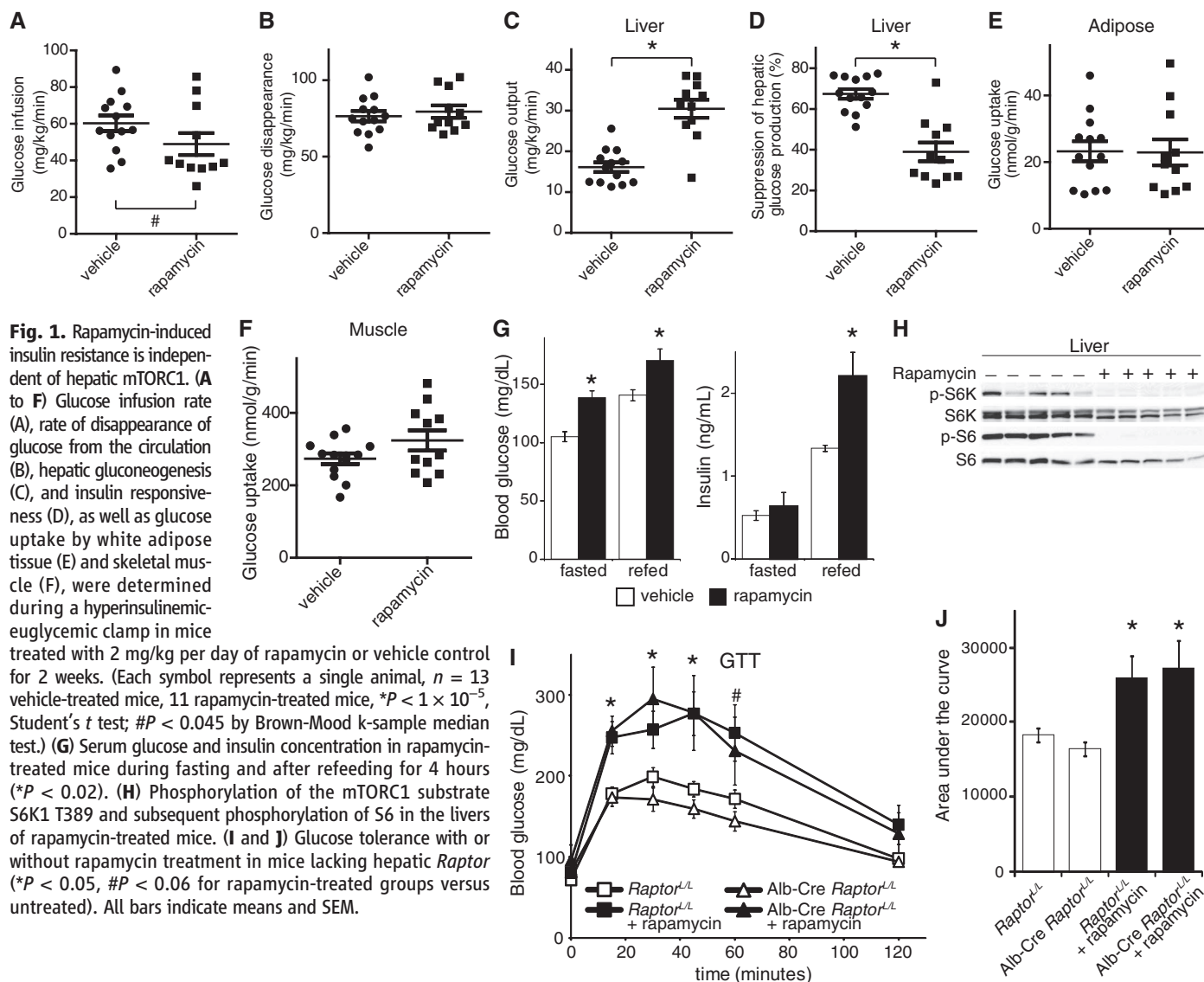
growth and differentiation, and mTORC2, which has a regulatory role in the insulin signaling cascade. Genetic attenuation of mTORC1 signaling promotes longevity in diverse organisms, including

Saccharomyces cerevisiae, *Caenorhabditis elegans*, and *Drosophila melanogaster*, and rapamycin-induced life span extension has been reported in *S. cerevisiae*, *D. melanogaster*, and mice (2–7). Moreover, deletion of the mTORC1 substrate S6 kinase 1 (S6K1) is sufficient to confer increased life-span in female mice (8). These findings strongly implicate mTOR in the regulation of mammalian longevity.

Calorie restriction (CR), a reduction in caloric intake while maintaining adequate nutrition, improves health and extends life-span in many organisms, including primates, through a mechanism that is not understood (9, 10). Yeast that lack the mTOR homolog *TOR1* have an extended replicative life span that is not further enhanced by CR, which suggests a common mechanism (4), but in flies, rapamycin increases life span beyond the maximum achieved by CR and thus may act through additional mechanisms (11). Improved glucose tolerance and insulin sensitivity are hallmarks of CR in mammals and a common feature of many models of increased longevity (12–16), al-

though mice lacking insulin receptor substrate (IRS) proteins 1 or 2 provide a notable counterexample (17, 18). One possible mechanism for enhanced insulin sensitivity during CR is reduced negative feedback through a signaling loop mediated by mTORC1 and S6K1 (19). Indeed, deletion of S6K1 is sufficient to improve insulin sensitivity and to extend life in mice (8), which suggests that rapamycin might act in a similar manner.

In contrast to the prediction that rapamycin would mimic the effects of CR or S6K1 deletion on glucose tolerance and insulin sensitivity, recent evidence suggests that chronic treatment with rapamycin impairs glucose homeostasis. Prolonged treatment with rapamycin leads to glucose intolerance in mice (20), as well as insulin resistance in rats (21, 22) and possibly humans (23, 24). We explored the mechanism by which rapamycin treatment impairs glucose homeostasis. Chronic rapamycin treatment, at approximately the same dose that was used to extend life span (~2 mg/kg per day), led to glucose intolerance and insulin resistance in both male and



female C57BL/6 mice (fig. S1, A and B). In rats, rapamycin-induced glucose intolerance results, in part, from increased hepatic gluconeogenesis (22). We found that rapamycin-treated mice had

increased expression of the gluconeogenic genes for phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6Pase) in their livers and had substantially impaired tolerance to pyr-

uvate, which indicated a failure to suppress gluconeogenesis (fig. S1, C and D).

We used a hyperinsulinemic-euglycemic clamp to measure insulin sensitivity in rapamycin-treated

Fig. 2. Disruption of mTORC2 in vivo after chronic rapamycin treatment. (A and B) Effects of rapamycin on phosphorylation of PKC α , Akt, and the SGK substrate NDRG1 in liver in response to refeeding (A) or insulin (B) after an overnight fast. (C and D) Effects of rapamycin on phosphorylation of PKC α and Akt in response to refeeding in white adipose tissue (C) and muscle (D). (E to G) Effects of rapamycin on the integrity of mTORC1 and mTORC2. mTOR was immunoprecipitated from liver (E), skeletal muscle (F), and white adipose tissue (G), followed by immunoblotting for Raptor and Rictor (subunits of mTORC1 and mTORC2, respectively).

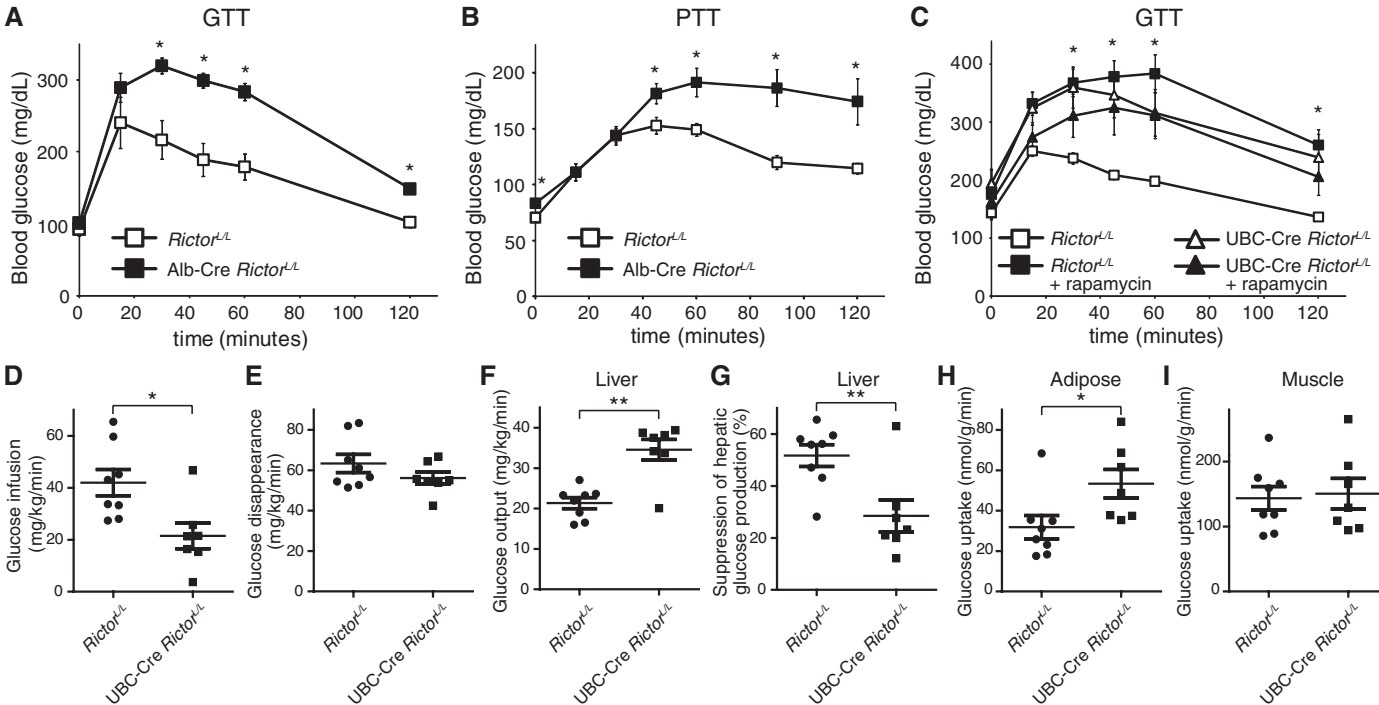
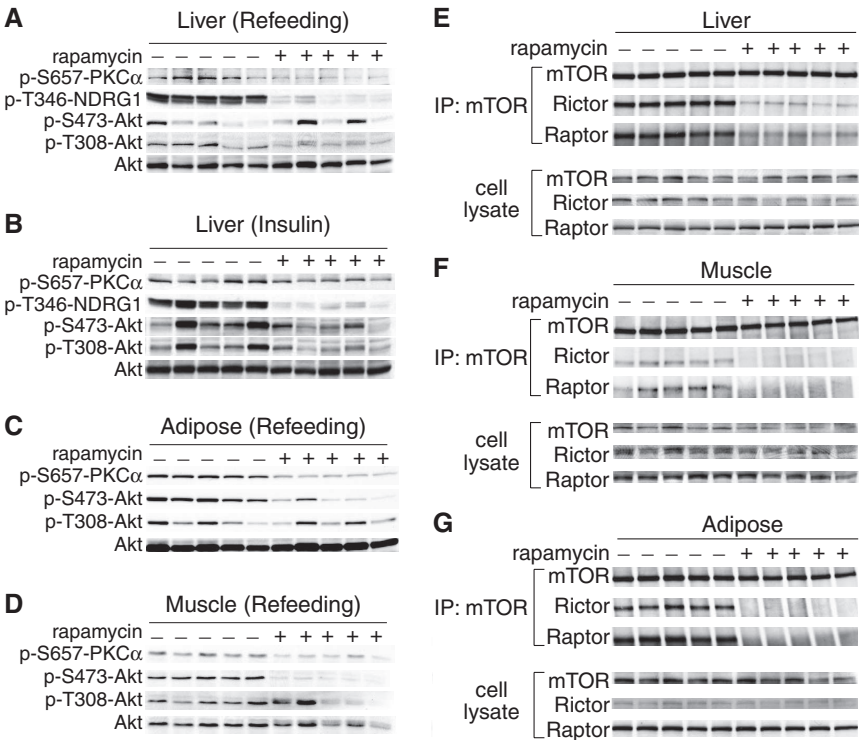


Fig. 3. Regulation of glucose homeostasis by mTORC2. (A) Glucose tolerance of Alb-Cre *Rictor*^{loxP/loxP} mice (**P* < 0.002). (B) Pyruvate tolerance of Alb-Cre *Rictor*^{loxP/loxP} mice (**P* < 0.03). (C) Effect of rapamycin on glucose tolerance of mice with whole-body deletion of Rictor fasted for 6 hours (**P* < 0.008 for all groups versus *Rictor*^{loxP/loxP}). (D to I) Glucose infusion rate (D), rate of disappearance of glucose from the circulation (E),

hepatic gluconeogenesis (F), and insulin responsiveness (G), as well as glucose uptake by white adipose tissue (H) and skeletal muscle (I), were determined during a hyperinsulinemic-euglycemic clamp in tamoxifen-treated ubiquitin-CreERT2 *Rictor*^{loxP/loxP} mice fasted for 6 hours (*n* = 8 *Rictor*^{loxP/loxP}, 7 UBC-Cre *Rictor*^{loxP/loxP}, ***P* < 0.008; **P* < 0.05). All bars indicate means and SEM.

mice (Fig. 1, A to F). Briefly, mice received a constant infusion of insulin, and radiolabeled glucose was coinfused as needed to prevent hypoglycemia. Calculating whole-body glucose uptake and hepatic glucose production, based on the fraction of labeled glucose in the circulation, revealed marked hepatic insulin resistance in rapamycin-treated mice (Fig. 1, C and D). In contrast, uptake of labeled glucose into white adipose and skeletal muscle was unaffected under clamp conditions (Fig. 1, E and F). In rapamycin-treated animals, plasma insulin was unchanged during fasting and higher after refeeding (Fig. 1G), which suggested an adaptive response to insulin resist-

ance rather than a defect in insulin production by pancreatic beta cells. These results indicate that hepatic insulin resistance is a major contributor to the impairment of glucose homeostasis by rapamycin and that white adipose tissue and skeletal muscle take up glucose normally in response to continuous insulin stimulation, despite inhibition of mTORC1 signaling to S6K1 in all three tissues (Fig. 1H and fig. S2, A and B).

We specifically disrupted hepatic mTORC1 signaling by expressing Cre under the control of the liver-specific albumin promoter in mice carrying a conditional allele of the mTORC1 subunit Raptor (regulatory associated protein of

mTOR) (fig. S2C). Alb-Cre *Raptor*^{loxP/loxP} mice, despite lacking hepatic mTORC1 activity, had normal glucose tolerance and remained responsive to rapamycin treatment (Fig. 1, I and J). These results indicate that disruption of hepatic mTORC1 signaling cannot account for the effects of rapamycin on glucose homeostasis. Although rapamycin is generally considered to be a specific inhibitor of mTORC1, extended treatment of cells with rapamycin can also physically disrupt mTORC2, independently from changes in protein expression in certain cell lines (25). We therefore examined mTORC2 signaling in livers, white adipose tissue, and muscles from rapamycin-treated

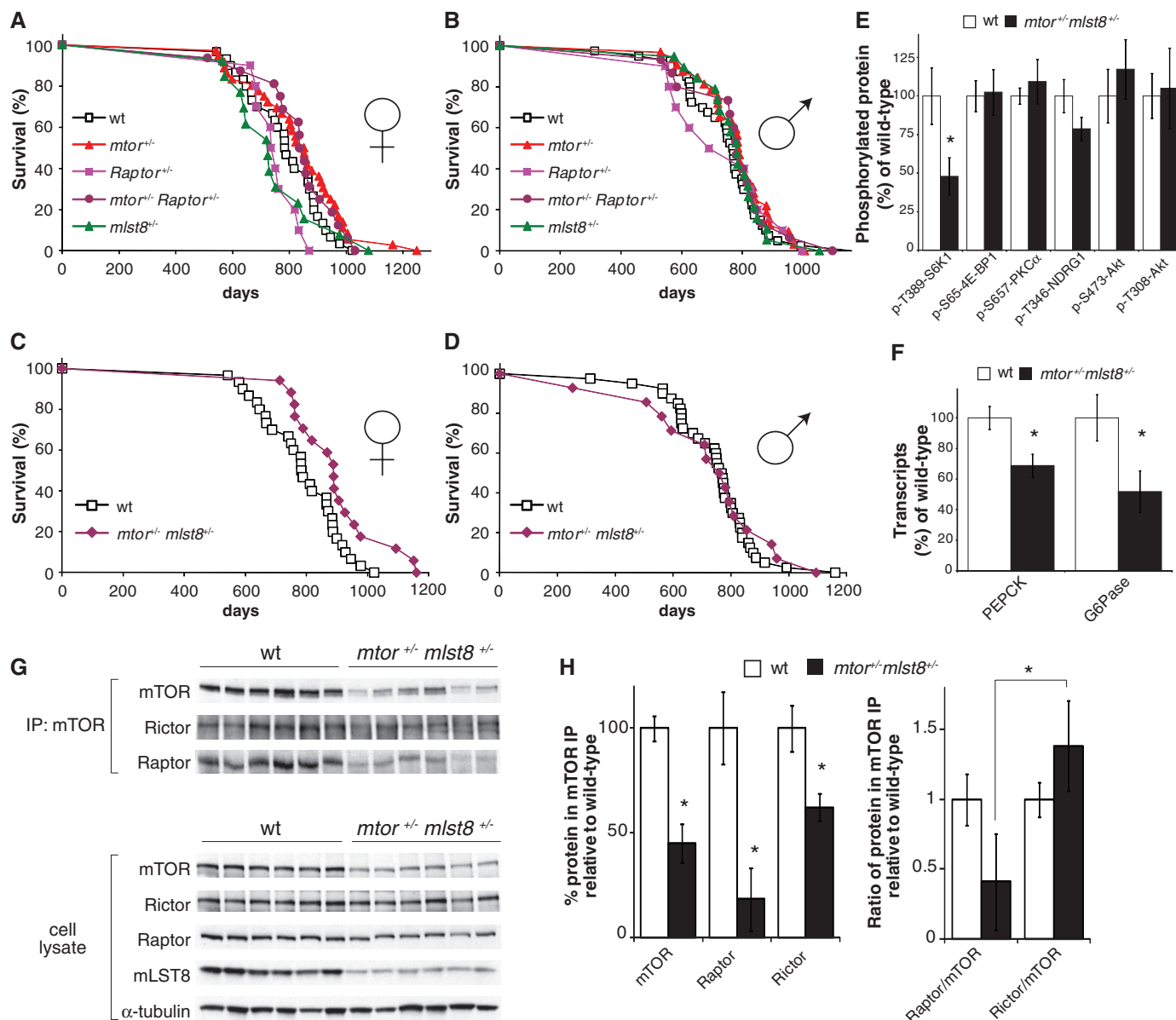


Fig. 4. Depletion of mTOR and mLST8 uncouples longevity from decreased glucose tolerance. (A and B) Kaplan-Meier plots showing life spans of female (A) and male (B) mice heterozygous for components of the mTOR signaling pathway. (C and D) Life-spans of female (C) and male (D) *mTOR*^{+/-} *mLst8*^{+/-} mice. Wild-type curves are repeated for comparison. (E) Quantification of phosphorylated proteins in female wild-type and *mTOR*^{+/-} *mLst8*^{+/-} livers after an

overnight fast and 45 min of refeeding ($n = 13$ wild-type versus 13 *mTOR*^{+/-} *mLst8*^{+/-} female mice, $*P < 0.03$). (F) Quantitative real-time polymerase chain reaction analysis of mRNA levels for PEPCK and G6Pase in the livers of young female wild-type and *mTOR*^{+/-} *mLst8*^{+/-} mice ($*P < 0.03$). (G and H) Immunoprecipitation of mTOR complexes reveals preferential loss of Raptor association in *mTOR*^{+/-} *mLst8*^{+/-} female mice ($*P < 0.02$). All bars indicate means and SEM.

mice. Mice were fasted overnight and then stimulated by refeeding or by intraperitoneal injection of insulin. Phosphorylation of mTORC2 substrates, including the protein kinases PKC α S657 and Akt S473, as well as the serum glucocorticoid-induced protein kinase (SGK) substrate (N-Myc downstream regulated 1) NDRG1 T346 (Fig. 2, A to D) was attenuated in all three tissues. To confirm that these effects resulted from mTORC2 disruption, we immunoprecipitated mTOR from the liver, white adipose tissue, and skeletal muscle of mice treated with rapamycin, and we visualized components of mTORC1 and mTORC2 by protein immunoblotting (Fig. 2, E to G). Chronic rapamycin treatment disrupted the association of mTOR with both Raptor (mTORC1) and Rictor (Raptor-independent companion of mTOR, mTORC2) in all three tissues. Therefore, direct disruption of mTORC2 is a second molecular mechanism that may contribute to the effects of rapamycin in vivo. Although rapamycin inhibited the phosphorylation of hepatic PKC α and the activity of SGK [as evidenced by decreased phosphorylation of the SGK substrate NDRG1 (26)] in animals that were fasted and refed for 45 min, the phosphorylation of hepatic Akt S473 was inhibited by rapamycin only in animals stimulated with insulin (49.7% of wild-type level, $P = 0.013$) (Fig. 2, A and B) (25). This may indicate that a small amount of residual mTORC2 is sufficient for basal, but not maximal, signaling to Akt.

To examine the effect of hepatic mTORC2 on glucose homeostasis, we generated mice that carried a Cre-excisable allele of the mTORC2 subunit *Rictor* and expressed Cre under the control of the albumin promoter (fig. S3A). Alb-Cre *Rictor*^{loxP/loxP} mice displayed pronounced glucose intolerance (Fig. 3A), but had a mild defect in an insulin tolerance test (fig. S3B), which likely reflected normal glucose uptake into skeletal muscle and adipose tissue. As with rapamycin-treated mice, Alb-Cre *Rictor*^{loxP/loxP} mice had impaired ability to suppress hepatic gluconeogenesis in a pyruvate tolerance test (Fig. 3B), had increased hepatic expression of G6Pase (fig. S4A), and did not show any overt defect in insulin secretion (fig. S4B). Thus, disruption of mTORC2 in the liver is sufficient to impair hepatic insulin sensitivity and is likely a major contributor to rapamycin-induced glucose intolerance.

Rapamycin disrupts mTORC2 in multiple tissues (Fig. 2, E to G). Deletion of *Rictor* in muscle impairs phosphorylation of Akt S473, although the effect on glucose homeostasis has not been described (27). Deletion of *Rictor* in adipose tissue leads to weight gain, hyperinsulinemia, and insulin resistance, with discordant findings reported as to the net effect on glucose tolerance (28, 29). To better understand the consequences of whole-body mTORC2 disruption, we created a strain of mice in which *Rictor* could be conditionally deleted in adult animals with a tamoxifen-inducible Cre (CreERT2) allele expressed under the control of the ubiquitin C promoter.

Consistent with the idea that rapamycin-induced hepatic insulin resistance is primarily mediated by mTORC2 disruption, there was no further effect on glucose homeostasis when ubiquitin C-CreERT2 *Rictor*^{loxP/loxP} mice were treated for 2 weeks with rapamycin (Fig. 3C). Note that Alb-Cre *Rictor*^{loxP/loxP} mice treated with rapamycin were hyperglycemic after 6 hours of fasting (fig. S6A) or refeeding (fig. S4B). This difference may reflect alterations in metabolism as a result of lifelong deficiency in hepatic Rictor. Nevertheless, rapamycin had no further effect on gluconeogenic gene expression, glucose tolerance, or pyruvate tolerance in Alb-Cre *Rictor*^{loxP/loxP} mice (figs. S4A and S6, B to E).

During a hyperinsulinemic-euglycemic clamp (Fig. 3, D to I), tamoxifen-treated ubiquitin C-CreERT2 *Rictor*^{loxP/loxP} mice had a decreased glucose infusion rate compared with tamoxifen-treated *Rictor*^{loxP/loxP} controls (Fig. 3D). As with the rapamycin-treated mice, the predominant defect in these animals under clamp conditions was hepatic insulin resistance, which resulted in increased glucose production (Fig. 3, F and G). Although the overall rates of glucose removal from the blood and uptake into skeletal muscle were not significantly affected, glucose uptake into white adipose tissue was increased (Fig. 3, H and I). Thus, Rictor appears to have a role in the regulation of glucose homeostasis in white adipose tissue, although chronic rapamycin treatment is not sufficient to elicit this phenotype (Fig. 1E), or the effect is offset by other rapamycin targets, such as mTORC1. Notably, *Rictor* was not completely excised in skeletal muscle, meaning that a role for mTORC2 in this tissue cannot be excluded at present. Thus, both rapamycin treatment and *Rictor* deletion induce clear hepatic insulin resistance, whereas the precise role of mTORC2 in regulating glucose uptake into other tissues remains to be fully defined.

Because the negative effects of rapamycin on glucose tolerance and hepatic insulin sensitivity appear to be mediated by mTORC2 disruption, whereas life-span extension has generally been presumed to result from mTORC1 inhibition, it may be possible to uncouple the beneficial and detrimental effects of mTOR inhibition. We therefore bred mice carrying only a single copy of *mtor*, *Raptor*, or *mlst8* (mammalian lethal with Sec13 protein 8), or double-mutant *mtor*^{+/-} *Raptor*^{+/-} and *mtor*^{+/-} *mlst8*^{+/-} mice and followed them as they aged.

There was no increase in life span in either female (Fig. 4A) or male (Fig. 4B) *mtor*^{+/-}, *Raptor*^{+/-}, *mlst8*^{+/-} or *mtor*^{+/-} *Raptor*^{+/-} mice (see also table S1). However, female *mtor*^{+/-} *mlst8*^{+/-} mice were long-lived, with a 14.4% increase in mean life span relative to wild type (Fig. 4C) (Dunnett's test, $P = 0.046$; Cox $P = 0.027$) (table S1). The longevity of male *mtor*^{+/-} *mlst8*^{+/-} mice was unaffected (Fig. 4D). Female *mtor*^{+/-} *mlst8*^{+/-} mice were not calorie-restricted through reduced food intake or increased energy expenditure and had normal body weights and levels of activity (fig. S7).

Consistent with the phenotypic effects, *mtor*^{+/-} *mlst8*^{+/-} mice exhibited a reduction of about 30 to 60% in the abundance of hepatic mTOR, Raptor, mLST8, and Rictor, whereas the expression of mTOR complex subunits was less affected in *Raptor*^{+/-} and *mtor*^{+/-} *Raptor*^{+/-} heterozygotes (fig. S8).

Consistent with the increased life span of female *mtor*^{+/-} *mlst8*^{+/-} mice, hepatic mTORC1 signaling, as assessed by S6K1 phosphorylation, was decreased by ~50% (fig. S9A and Fig. 4E). Although both mTOR and mLST8 are subunits that are shared between the two mTOR complexes, there was no significant change in mTORC2 signaling as assessed by Akt, PKC α , or NDRG1 phosphorylation. Phosphorylation of 4E-BP1 was not changed in the livers of female *mtor*^{+/-} *mlst8*^{+/-} mice; however, we observed decreased phosphorylation of 4E-BP1 in mouse tail fibroblasts in vitro (fig. S10, A and B), which suggested that both S6K and 4E-BP1 signaling may be affected. Hepatic S6K1 T389 phosphorylation was also lower in male *mtor*^{+/-} *mlst8*^{+/-} mice than in controls ($P < 0.08$) (fig. S10C).

Female *mtor*^{+/-} *mlst8*^{+/-} mice had normal glucose tolerance (fig. S9B), insulin sensitivity (fig. S11A), and fasting insulin levels (fig. S11B), which further indicated that mTORC2 signaling was intact. Moreover, female *mtor*^{+/-} *mlst8*^{+/-} mice had decreased expression of PEPCK and G6Pase, which suggested that control of hepatic glucose production may even have been improved (Fig. 4H). Although absolute amounts of both mTORC1 and mTORC2 decreased in the livers of female *mtor*^{+/-} *mlst8*^{+/-} mice, the ratio of Raptor to mTOR declined more than the ratio of Rictor to mTOR (Fig. 4, I and J). Thus, mTORC2 appears to compete more effectively for a limiting amount of the mTOR catalytic subunit.

Glucose tolerance (area under the curve) was not significantly affected in young animals from any of the strains heterozygous for mTORC1 components, although the glucose tolerance of male *Raptor*^{+/-} mice improved with age (fig. S11, C and D). The increased life span and normal glucose tolerance of female *mtor*^{+/-} *mlst8*^{+/-} mice, and the unchanged life-span despite increased glucose tolerance in male *Raptor*^{+/-} mice are both indicative that the effects of rapamycin on longevity and glucose homeostasis can be uncoupled.

Our results suggest that rapamycin extends life span, at least in part, by inhibition of mTORC1 and despite impairing glucose homeostasis by means of disruption of mTORC2 signaling. Female *mtor*^{+/-} *mlst8*^{+/-} mice, which had selectively impaired mTORC1 signaling, had increased longevity without overt changes in insulin signaling. Our findings emphasize that mTORC2 disruption profoundly affects metabolism and may be relevant to the pathogenesis of type 2 diabetes and the metabolic syndrome. *S6K1*^{-/-} mice, which, like *mtor*^{+/-} *mlst8*^{+/-} mice, exhibit female-specific life-span extension, are resistant to diet-induced weight gain and insulin resistance (8). However,

mtor^{+/-} *mlst8*^{+/-} mice did not display these phenotypes (fig. S12), which demonstrated that factors related to energy balance can also be uncoupled from longevity. Specific disruption of mTORC2 extends life span in worms fed a nutrient-rich diet (30). If this effect is conserved in mammals, disruption of mTORC2 may contribute to the pro-longevity effect of rapamycin. Nevertheless, our present findings suggest that specific inhibitors of mTORC1 might provide many of the benefits of rapamycin on health and longevity, while avoiding side effects that currently limit its utility.

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Supporting Online Material

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Materials and Methods

Figs. S1 to S12

Table S1

References (31–41)

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hnRNP C Tetramer Measures RNA Length to Classify RNA Polymerase II Transcripts for Export

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Specific RNA recognition is usually achieved by specific RNA sequences and/or structures. However, we show here a mechanism by which RNA polymerase II (Pol II) transcripts are classified according to their length. The heterotetramer of the heterogeneous nuclear ribonucleoprotein (hnRNP) C1/C2 measures the length of the transcripts like a molecular ruler, by selectively binding to the unstructured RNA regions longer than 200 to 300 nucleotides. Thus, the tetramer sorts the transcripts into two RNA categories, to be exported as either messenger RNA or uridine-rich small nuclear RNA (U snRNA), depending on whether or not they are longer than the threshold, respectively. Our findings reveal a new function of the C tetramer and highlight the biological importance of RNA recognition by the length.

Different RNA species are exported from the nucleus in distinct complexes (*I*), whose protein compositions can regulate downstream gene expression (2, 3). Among them, spliceosomal U small nuclear RNA (snRNA) and mRNA precursors are similarly transcribed by polymerase II (Pol II) and therefore initially acquire m⁷G-cap structures, to which the common factor, cap-binding complex (CBC), binds (*I*). However, their export complex assemblies are subsequently different. In U snRNA export,

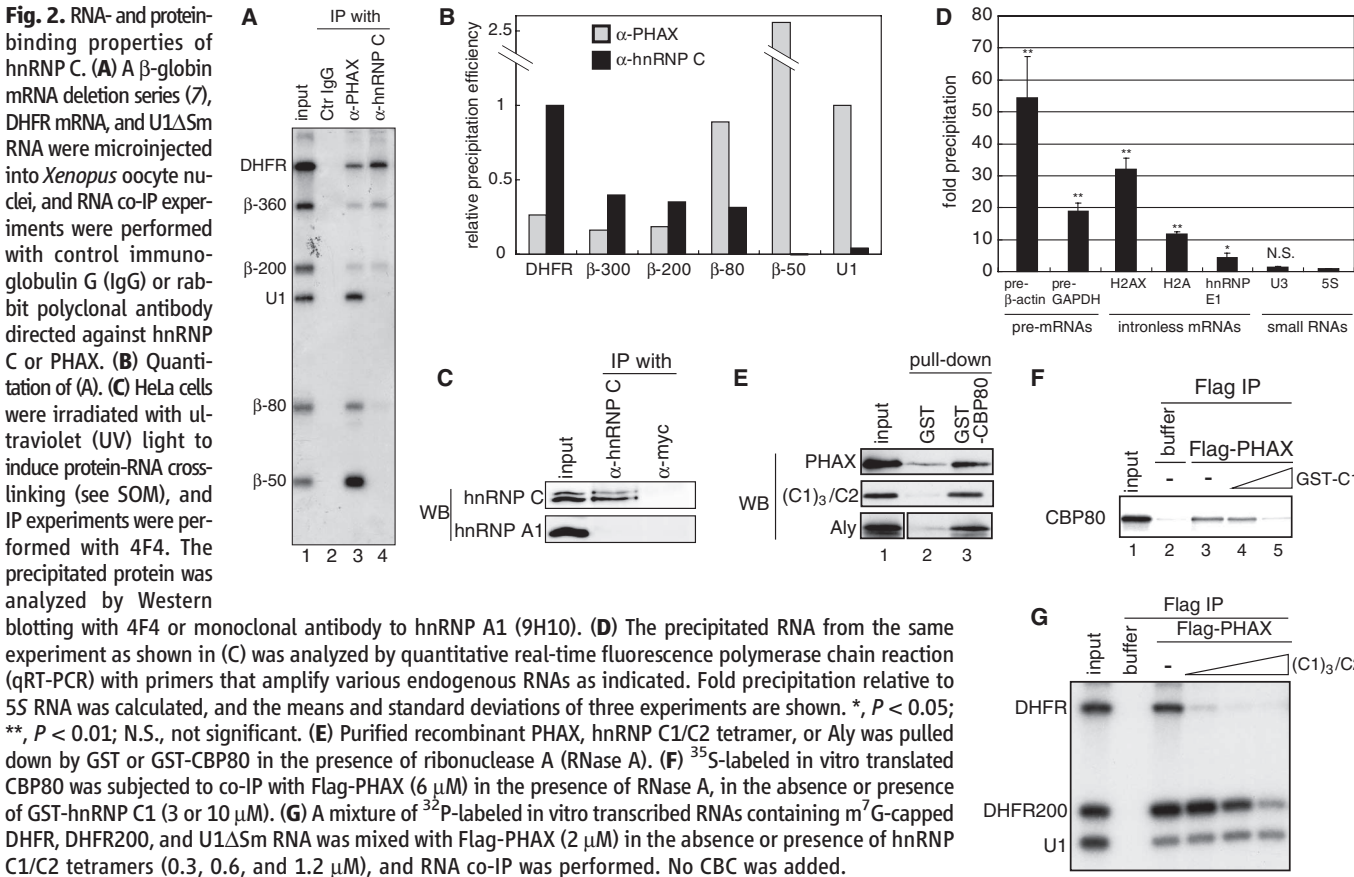
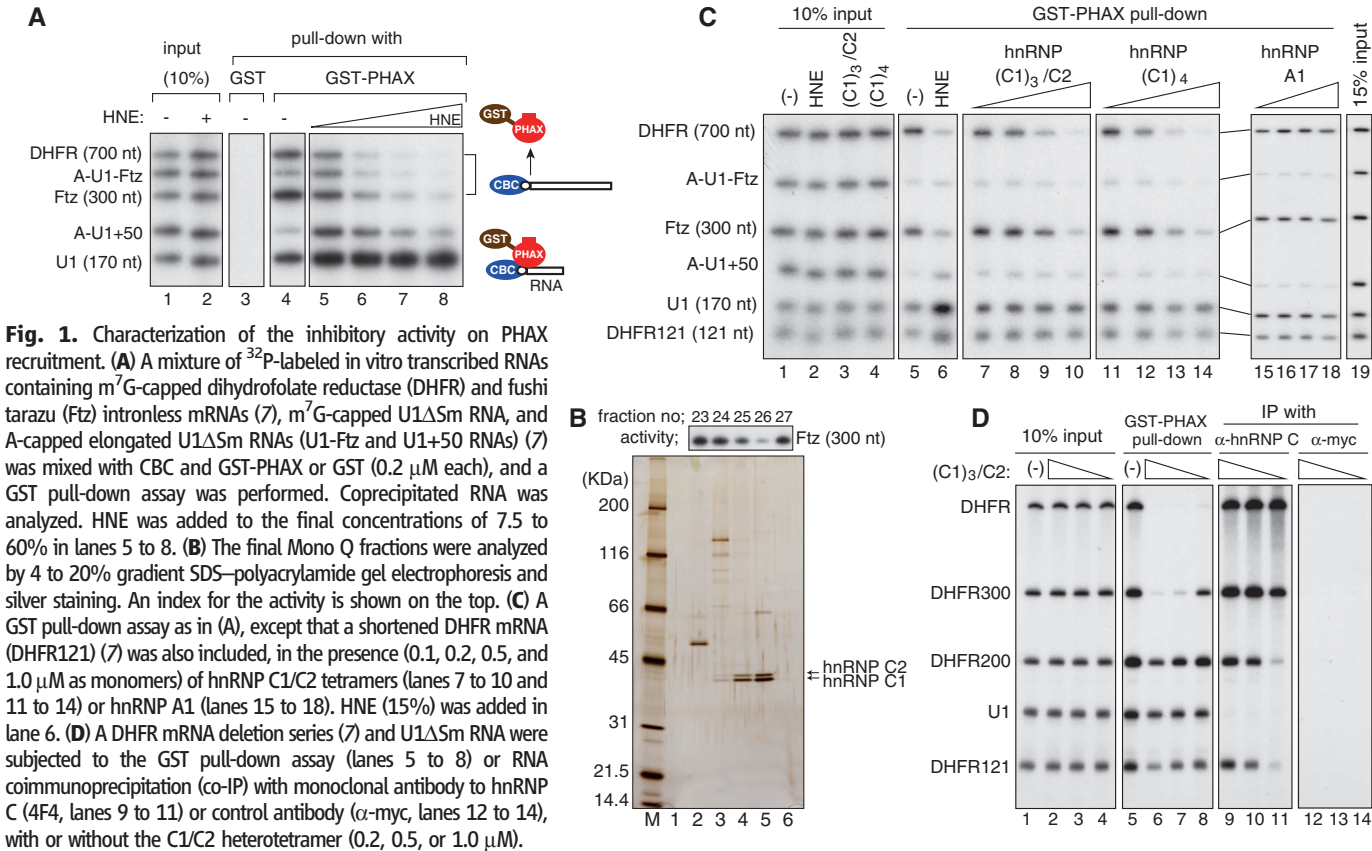
an adaptor protein PHAX binds to both CBC and RNA near the cap, and PHAX subsequently recruits CRM1-RanGTP (4). In contrast, in bulk mRNA export, RNA-binding adaptor proteins, such as Aly/REF, are first recruited to the RNA, often in the context of larger protein complexes, the transcription/export (TREX) complex, and/or the exon junction complex (EJC). These adaptor proteins subsequently recruit the major mRNA export receptor TAP/NXF1 to the RNA (*I*, 5). The mRNA export complex normally lacks U snRNA export factors, such as PHAX (6, 7). Thus, these two RNAs, although they share some similarities, must have distinguishing features that are recognized by the cellular RNA export machinery. One of them is related to the RNA length (6–8). m⁷G-capped transcripts take either the U

snRNA or mRNA export pathway, depending on their lengths rather than on their sequences. The threshold length for this phenomenon is around 200 to 300 nucleotides (nt), except for highly structured RNA regions, which are incompetent for exerting this RNA length effect (6–8). We developed an in vitro system that recapitulates the remodeling of RNA-protein complexes according to RNA length to address this issue. In vitro transcribed RNAs of various lengths were mixed with recombinant CBC and glutathione S-transferase (GST)–PHAX fusion protein, and a GST pull-down assay was used to examine the formation of the trimeric complex of RNA, CBC, and PHAX (Fig. 1A). PHAX associated with all the m⁷G-capped RNAs regardless of their length, indicating that the in vivo situation was not recapitulated in this purified system (Fig. 1A, lane 4). In contrast, when an aliquot of a HeLa nuclear extract (HNE) was added to the system, PHAX binding to the longer RNAs was specifically inhibited (Fig. 1A, lanes 5 to 8). This activity did not require adenosine triphosphate hydrolysis (fig. S1). PHAX binding to some RNAs, like U1 and A-capped U1+50, was stimulated by HNE. This activity was distinct from the inhibitory activity on PHAX recruitment and will be discussed elsewhere.

This inhibitory activity on PHAX recruitment was biochemically purified from HNE [see supporting online material (SOM)]. The final purified fractions contained two major proteins whose appearance correlated with the activity (Fig. 1B and fig. S2). Mass spectrometric analyses revealed that these proteins corresponded to hnRNP C1 and C2 proteins. hnRNP C1 and C2 are nuclear RNA-binding proteins that form a 3:1 heterotetramer

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(9, 10), which cotranscriptionally associates with the majority of nascent mRNA transcripts, mostly in a nonspecific and contiguous manner (11, 12).

Recombinant hnRNP C proteins formed hetero- and homotetramers (fig. S3) (10), and the recombinant C1/C2 heterotetramer and the C1 homotetramer—but not hnRNP A1, which also binds RNA with high affinity—specifically inhibited PHAX binding to longer RNAs (Fig. 1C). The hnRNP C tetramers preferentially bound to longer RNAs in vitro (Fig. 1D) and in *Xenopus* oocyte nuclei (Fig. 2A and B). This tetramer binding to RNA excluded PHAX binding (Fig. 1D and Fig. 2, A and B). In HeLa cell lysates, hnRNP C proteins associated with cellular pre-mRNAs as well as with intronless mRNAs, but not with small RNAs (Fig. 2, C and D), and PHAX binding and hnRNP C binding were mu-

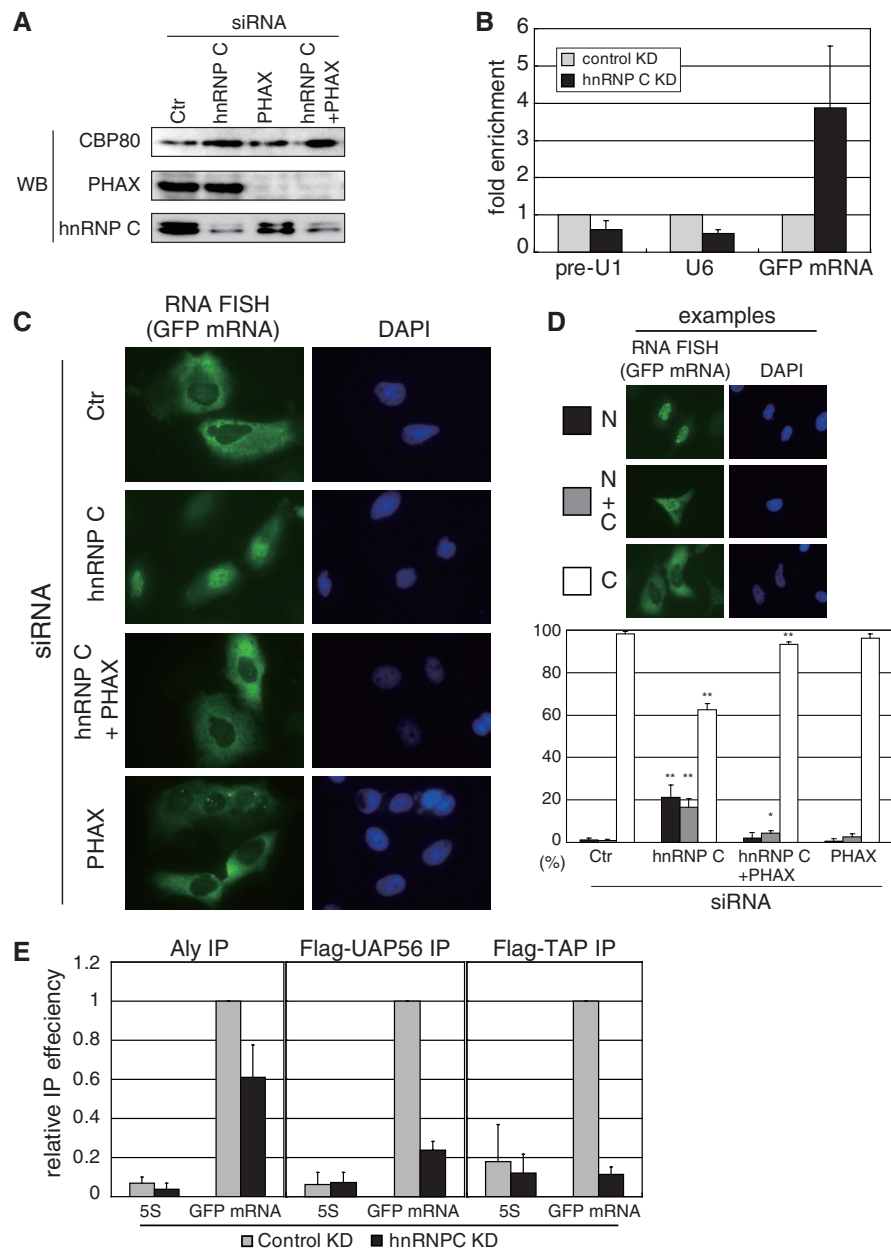
tually exclusive (fig. S4). A single C tetramer has been previously shown to occupy a 230 to 240 nt RNA region (11), which is consistent with the threshold RNA length in our study.

PHAX is recruited near the cap structure by simultaneously interacting with CBP80, the 80-kD subunit of CBC, and the cap-proximal RNA region (4). However, the C tetramer also interacted with not only RNA but also CBP80 (Fig. 2E and fig. S5), thereby competitively inhibiting PHAX binding to both CBP80 (Fig. 2F) and RNA (Fig. 2G). This is probably the mechanism by which the C tetramer inhibits PHAX recruitment. This mechanism may also stabilize the CBC binding and ensure that the contiguous RNA binding of the C tetramer (11, 12) starts from the cap without a gap.

The expression of the hnRNP C proteins in HeLa cells was successfully knocked down by

~80% by small interfering RNA (siRNA) (Fig. 3A). The association of PHAX with intronless green fluorescent protein (GFP) reporter transcripts [~970 nt, excluding the poly(A) tail] increased by a factor of 4 in the hnRNP C knocked-down (KD) cells compared with that in the control cells (Fig. 3B). The GFP mRNA accumulated in the nuclei of the hnRNP C KD cells, whereas the mRNA was predominantly cytoplasmic in control cells (Fig. 3, C and D, for quantitation), suggesting that mRNA export is inhibited when the level of hnRNP Cs is severely reduced. This is due to a reduction in the recruitment of mRNA export factors (Fig. 3E). Aly/REF was recruited but was not functional in recruiting TAP/NXF1. In contrast, if PHAX was knocked down simultaneously with the hnRNP C proteins, mRNA export was restored (Fig. 3, C and D, and fig. S8),

Fig. 3. Knockdown of hnRNP C results in the inhibition of intronless GFP mRNA export. **(A)** HeLa cells were transfected with siRNAs as indicated, and Western blotting was performed with antibodies directed against CBP80, PHAX, or hnRNP C. **(B)** HeLa cells, transfected with control siRNA or siRNA against hnRNP C, were transfected with an intronless GFP reporter plasmid (pcDNA3-GFP). At 1 hour after plasmid transfection, when the majority of the GFP transcripts was still in the nucleus (fig. S6), the cells were lightly fixed with formaldehyde. IP was then performed with affinity-purified polyclonal antibody to PHAX, and the coprecipitated RNA was analyzed by qRT-PCR with the primers for endogenous U1 RNA precursor (pre-U1), U6, or exogenous GFP mRNA. The means and standard deviations of three experiments are shown. **(C)** HeLa cells, transfected as in (B), were subjected to RNA-FISH with a GFP probe at 16 hours after the plasmid transfection. **(D)** Quantitation of (C). The cells with a nuclear (N), nuclear+cytoplasmic (N+C), or cytoplasmic (C) RNA signal were counted, and their percentages were calculated. The means and standard deviations of three experiments are shown. *, $P < 0.05$; **, $P < 0.01$. **(E)** RNA-IP was performed with HeLa cells (for the IP with endogenous Aly), or HeLa cells transiently expressing Flag-TAP (for the IP with TAP), or stably expressing Flag-UAP56 (for the IP with UAP56). These HeLa cells were transfected as in (B). After 14 hours, HeLa cells were either irradiated with UV light for the IP with antibody to Aly (11G5) or lightly fixed with formaldehyde for other IPs with antibody to Flag (M2). In this KD condition, the expression of the three proteins was not affected (fig. S7). The precipitated RNA was analyzed by qRT-PCR, and the relative IP efficiencies were normalized by the average amounts of GFP mRNA per nucleus, determined by quantifying the RNA-FISH images with Image J software ($n > 75$).



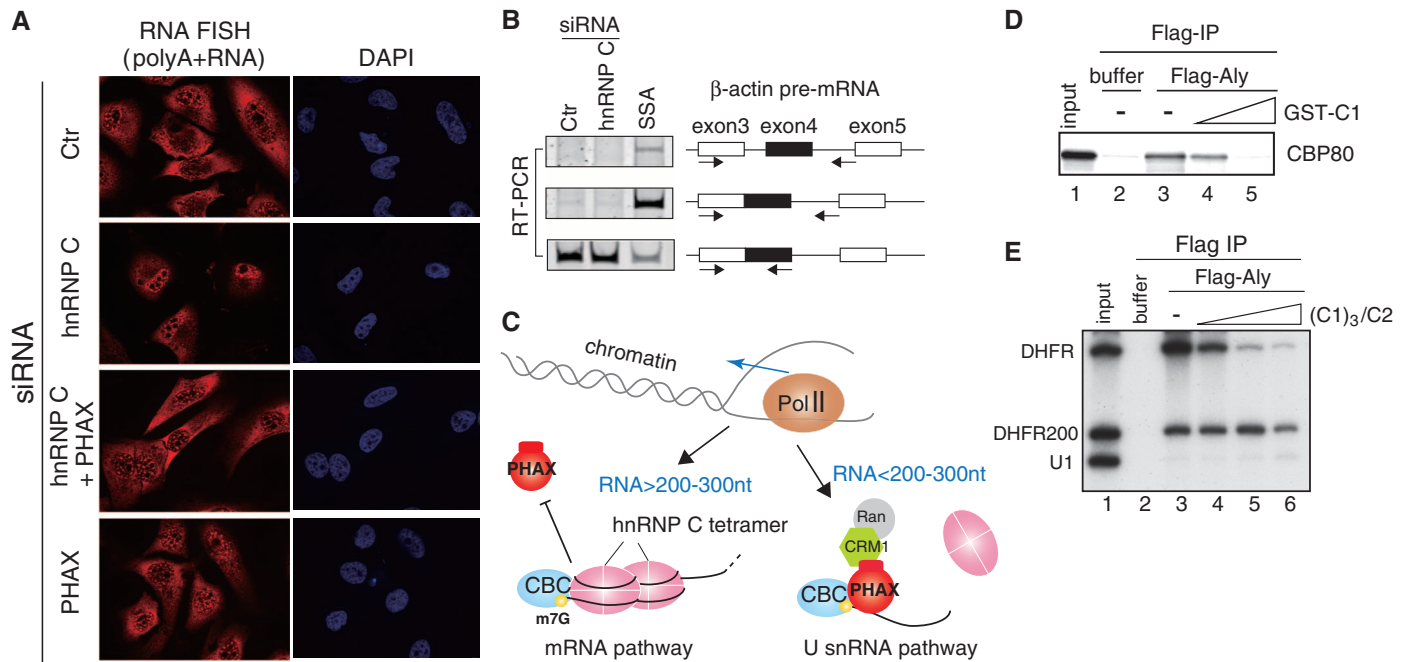


Fig. 4. HnRNP C has a general role in mRNA export. **(A)** RNA-FISH was performed as in Fig. 3C except that an oligo(dT) probe was used. See fig. S11 for the results produced with different siRNA against hnRNP C. **(B)** RT-PCR was performed to amplify three regions of the endogenous β -actin transcripts. **(C)**

Model of the role of the hnRNP C tetramer in sorting Pol II transcripts. **(D)** Protein co-IP assay as in Fig. 2F, except that purified recombinant Flag-Aly (2 μ M) was used. **(E)** RNA co-IP assay as in Fig. 2G, except that Flag-Aly (0.25 μ M) was used.

whereas inhibiting CRM1 binding by leptomycin B had no such rescue effect (fig. S9). When a GFP reporter construct with an intron was used, similar results were obtained (fig. S10). Thus, the prevention of PHAX binding per se to Pol II transcripts by the hnRNP C tetramer is essential for efficient export of spliced and intronless mRNAs.

The distribution of endogenous bulk poly(A)+RNAs was next examined with RNA fluorescence in situ hybridization (RNA-FISH), using an oligo(dT) probe, and similar results were obtained (Fig. 4A). Pre-mRNA splicing was not affected by the hnRNP C knockdown, as demonstrated by the splicing of introns 3 and 4 of the β -actin gene (Fig. 4B). Therefore, we conclude that the hnRNP Cs function in the export of endogenous mRNAs by precluding any potential interference by PHAX.

Our model of the role of hnRNP C proteins in RNA sorting is shown in Fig. 4C. Immediately after the onset of Pol II transcription, the transcript acquires an m⁷G-cap structure, with which CBC associates cotranscriptionally (13). At this point, the machinery still does not know whether the transcript will be long or short. If the nascent transcript emerging from the chromatin template becomes longer than 200 to 300 nt, the hnRNP C1/C2 heterotetramer binds to the transcript and the cap-bound CBC. PHAX recruitment is then inhibited because the C tetramer competitively inhibits PHAX binding to both CBC and RNA (Fig. 2, F and G), and thus the transcript is committed to the mRNA export pathway. If the tran-

script is shorter than the threshold, on the other hand, the heterotetramer cannot stably bind and the RNA is committed to the U snRNA export pathway.

Because hnRNP C proteins do not shuttle between the nucleus and cytoplasm (14), the heterotetramers must be released from the mRNA transcripts in the nucleus. Aly/REF, an adaptor protein for mRNA export, is recruited by interacting with both CBC and RNA (Fig. 2E) (15, 16). HnRNP C competitively inhibits both of these interactions in vitro (Fig. 4, D and E). Therefore, the cap-proximal C tetramer must be replaced by Aly/REF, probably in the context of TREX complex and/or EJC, in the course of the formation of export-competent messenger RNP (fig. S12). This should also prevent rebinding of PHAX. Thus, three factors—PHAX, hnRNP C, and Aly/REF—compete with each other for the interaction with the cap-bound CBC and the cap-proximal RNA region.

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Supporting Online Material

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Figs. S1 to S13
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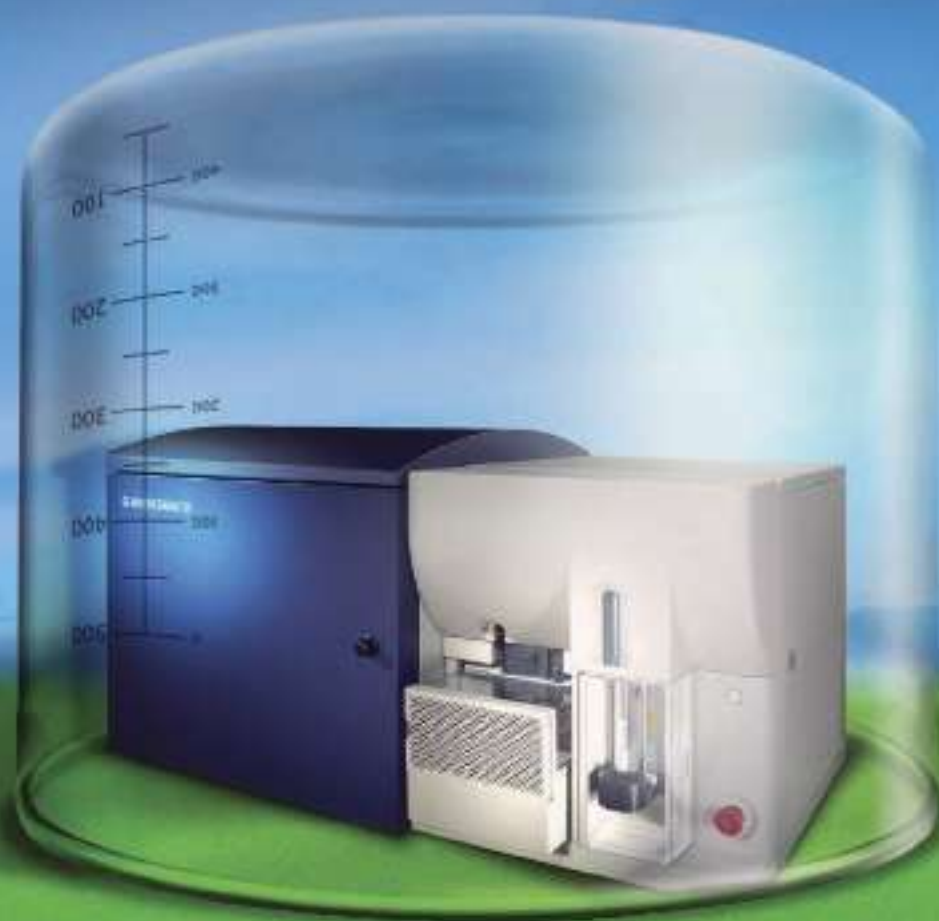
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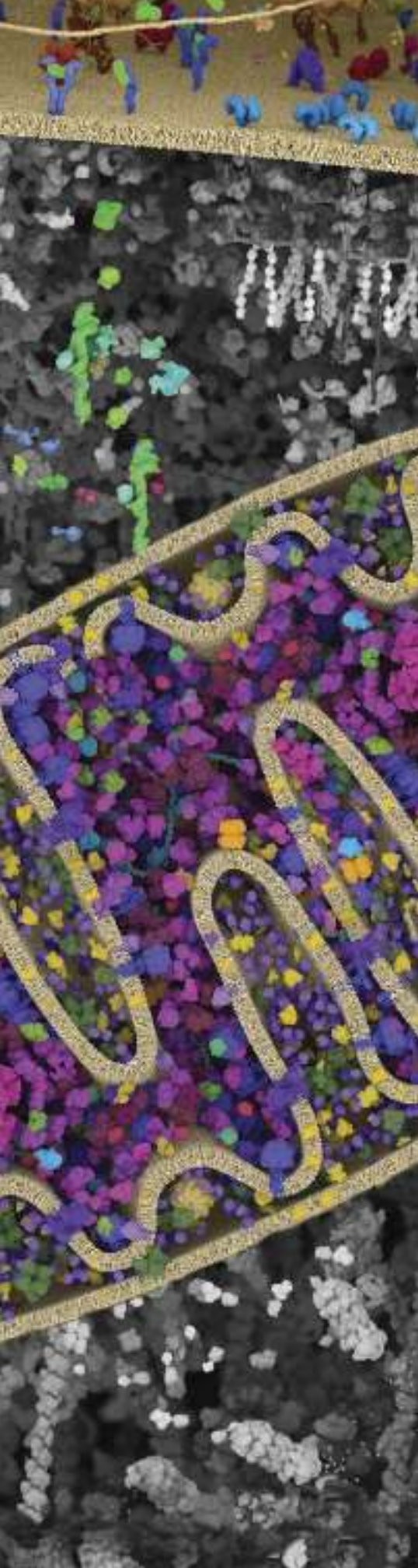
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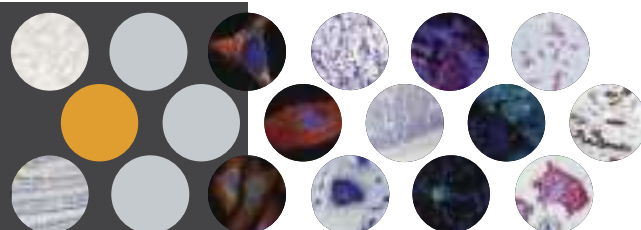
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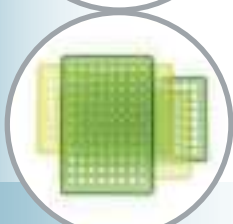
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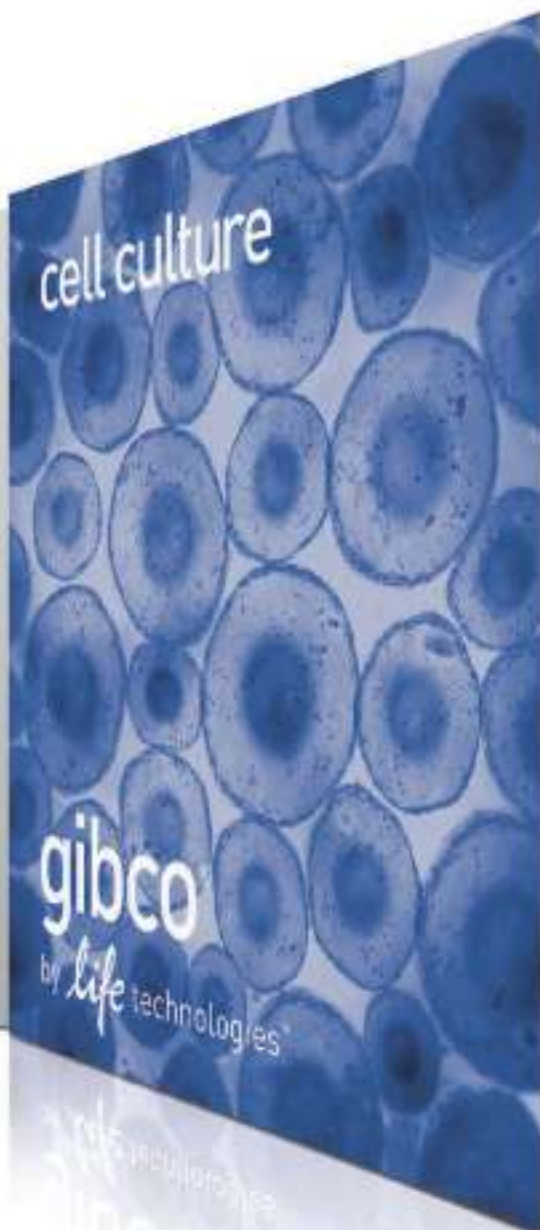
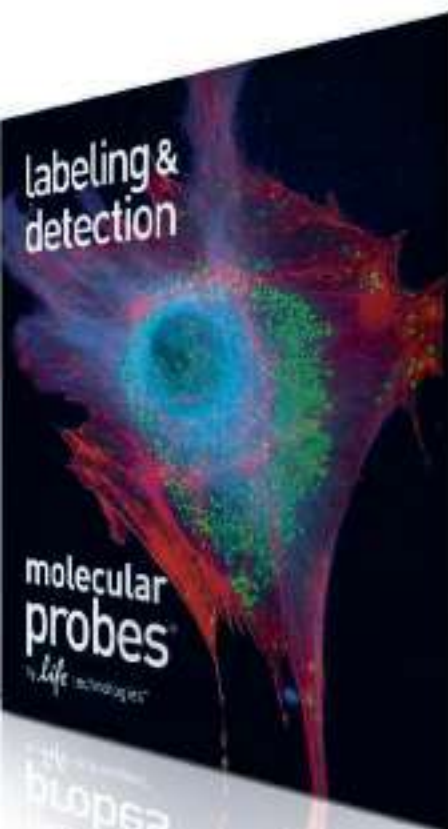
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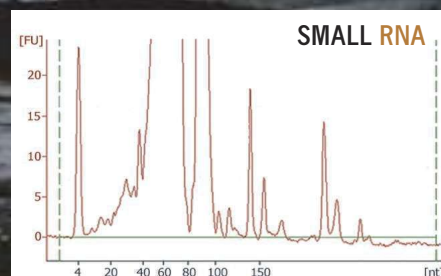
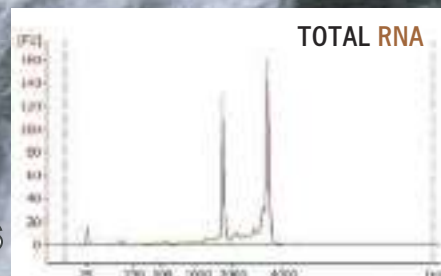
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* Piotr Chomczynski, US patent 2010

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Call for Symposium Proposals

Symposium proposals for the **2013 AAAS Annual Meeting** are now being solicited. To submit a proposal, visit www.aaas.org/meetings. **The deadline for submission is Thursday, 26 April 2012.**

The Beauty and Benefits of Science

The theme for the meeting points to the “unreasonable effectiveness” of the scientific enterprise in creating economic growth, solving societal problems, and satisfying the essential human drive to understand the world in which we live.

The phrase, “unreasonable effectiveness,” was coined in 1960 by physicist Eugene Wigner,

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Student Poster Competition

The competition recognizes the individual efforts of students actively working toward an undergraduate, graduate, or doctoral degree. **Online entries will be accepted beginning 14 May 2012.**

For information about exhibits and sponsorships, contact meetings@aaas.org.



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The Ingenuity Variant Analysis is a web application designed to help researchers studying human disease rapidly identify causal variants from human resequencing data. Researchers can quickly and reliably take a list of millions of variants down to the most compelling set of variants for follow-up study and analysis. Ingenuity Variant Analysis supports individual whole genome or exome studies with thousands of samples without deleting data sets. Unlike other software products that focus only on called and annotated variants, Ingenuity Variant Analysis streamlines the annotation and prioritizing of all variants through rich biological interpretation and analysis. Its unique combination of filtering, analytics, and richly annotated content allows researchers to identify and prioritize variants by drilling down to a small, targeted subset of compelling variants based both upon published biological evidence and the researcher's own knowledge of disease biology.

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A new custom assay design service for high-sample throughput amplicon resequencing on Illumina platforms is now available. The Access Array Target-Specific Primers, when used with the Fluidigm Access Array System, allow for fast, simple, and inexpensive preparation of up to 480 amplicons per sample at a time. This capability is ideal for analysis of large sample sets across focused genomic regions to better understand human genetic variation. Access Array Target-Specific Primers allow researchers to quickly and specifically amplify their target of interest, incorporate sample-specific barcodes, and add sequencer-specific adaptors—all at the same time. The Fluidigm Access Array System prepares amplicons for resequencing without the need for any additional library preparation and can operate with any next generation sequencer (NGS) on the market. In addition, using the Access Array Barcode Libraries, researchers can test the same amplicon pools to cross-validate sequencing results across different NGS platforms.

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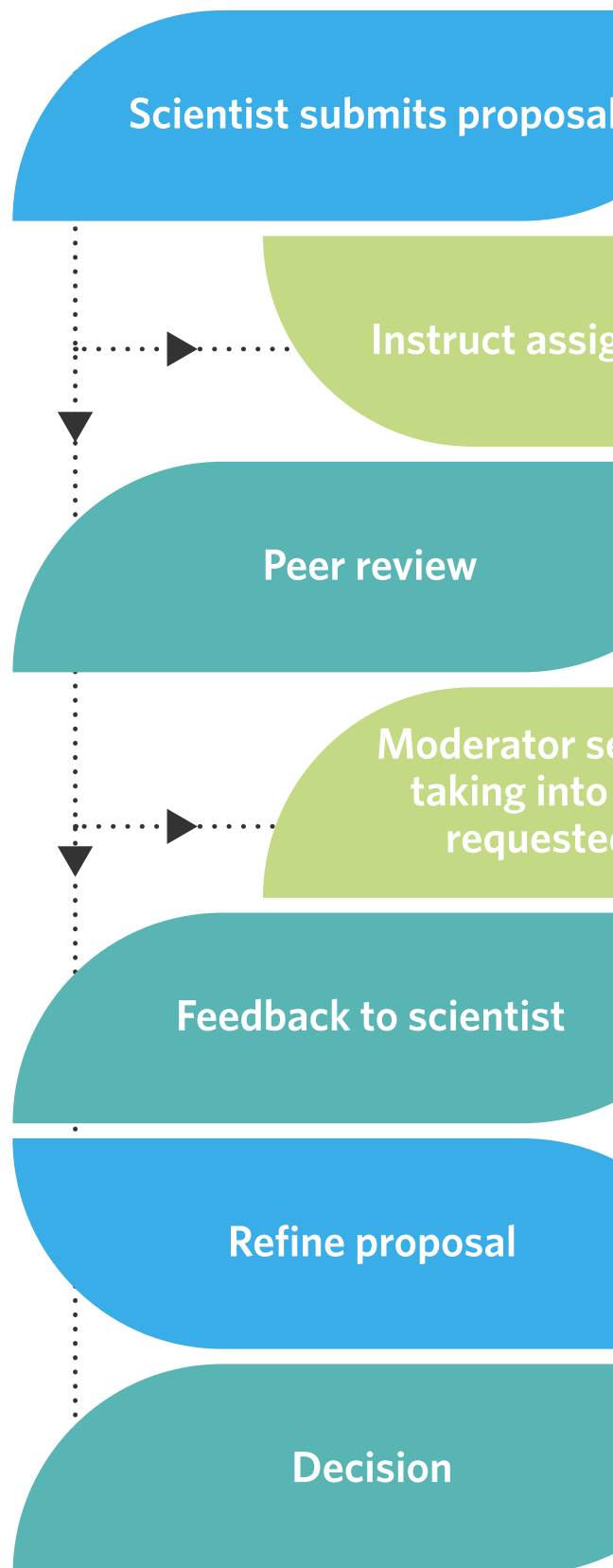
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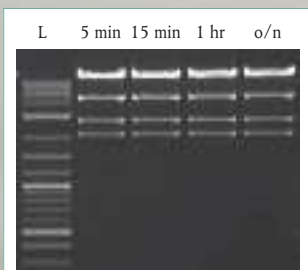
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Science Careers

From the journal *Science*



CMU
CENTRAL MICHIGAN
UNIVERSITY

BASIC SCIENCE FACULTY MULTIPLE POSITIONS ASSISTANT/ASSOCIATE/FULL PROFESSOR

The Central Michigan University College of Medicine (CMED) seeks highly qualified faculty for multiple tenure-track and fixed term positions. Successful candidates will assume a role in the development and implementation of a highly integrated, cross disciplinary, clinical presentation and inquiry-based medical school curriculum.

Positions will be at Assistant/Associate/Full Professor rank, depending on credentials, and are available immediately. Start dates negotiable. Applications are being sought for teaching expertise in all disciplines of the basic sciences with immediate needs in Anatomy, Pharmacology, and Microbiology.

Requires doctoral degree, strong commitment to innovative approaches to education and learning, ability or commitment to facilitate interactive learning in large and small group settings, experience with independent design and conduct of research consistent with discipline(s). See www.jobs.cmich.edu for complete list of qualifications, including requirements for advanced rank.

While all areas of research will be considered, collaborative opportunities in basic research exist in neuroscience, mitochondrial biology, and cancer vaccines. Additional desired areas of research include: education, comparative effectiveness, health services, and clinical trials and outcomes.

Established in 1892, CMU offers more than 200 academic programs at the undergraduate through doctoral levels, including several that are nationally recognized. CMU's College of Medicine will welcome its inaugural class in summer 2013. The innovative medical school curriculum is being designed to prepare students for practice in mid- to northern Michigan and the Upper Peninsula, with particular attention to primary care needs in the region. For more information, visit <http://www.cmich.edu/med>.

Review of applications begins immediately and continues until filled. **Applications must be submitted through an on-line process at: www.jobs.cmich.edu.** Electronically attach a letter of application, CV, evidence of teaching ability, statement of teaching philosophy, statement of research/scholarly interest, and a list of three professional references, including phone numbers & email addresses. Direct questions to Rebecca Messing, Search Committee Coordinator, Spenc1r1@cmich.edu, 989-774-7862.

CMU, an AA/EQ Institution, strongly and actively strives to increase diversity within its community (see www.cmich.edu/aaeo).



REGIONAL CENTRE FOR BIOTECHNOLOGY
an institution of education, training & research

(Established by the Dept. of Biotechnology, Govt. of India under the auspices of UNESCO)

Corrigendum to Adv. No.1/12

YOUNG VISITING SCIENTISTS AWARD

The Regional Centre for Biotechnology (RCB) invites applications for Young Visiting Scientists Award from the eligible candidates residing in the regional member countries of UNESCO (other than India). The Young Visiting Scientists Award will be initially for one year extendable for another year on merit basis.

The Regional Centre for Biotechnology (RCB) has been established by the Department of Biotechnology, Govt. of India under an agreement with UNESCO as its Category II Centre. It is designed to be a Centre of excellence in biotechnology with intimate contributions from the countries of the region and academic institutions from the rest of world. It provides a meeting place where innovation, enterprise and industrial development will germinate.

The objective of the Young Visiting Scientists Award is to identify and nurture outstanding young scientists with innovative ideas and desirous of pursuing research in frontier areas of biotechnology related but not limited to structural, systems and synthetic biology, nano science & technology, cellular and molecular biology, disease biology and data-intensive discovery research under the mentorship of senior faculty at the Regional Centre for Biotechnology. Young Scientists below the age of 35 having PhD degree in any discipline of natural science are eligible to apply. The candidates selected for the Young Visiting Scientists Award will be given one to and fro international fare, leased residential accommodation and medical coverage in addition to the Award of Rs. 40,000/- per month. The Awardee shall not be entitled to draw any other remuneration, fellowship or salary. They will have to obtain inter-governmental clearances and international passport and visa on their own. However necessary documents will be provided to them for the purpose.

Interested candidates may submit their applications in the prescribed format which can be downloaded from the website www.rcb.res.in, to the **Registrar, Regional Centre for Biotechnology, 180, Udyog Vihar, Phase- I, Gurgaon-122016, Haryana (India)**. The date of submission of application has been extended up to **April 30, 2012**. A copy of the duly filled in application form should also be sent on the email ID registrar@rcb.res.in.

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EMBL offers a highly collaborative, uniquely international culture. It fosters top quality, interdisciplinary research by promoting a vibrant environment consisting of young, independent researchers with access to outstanding graduate students and postdoctoral fellows. EMBL is an inclusive, equal opportunity employer offering attractive conditions and benefits appropriate to an international research organisation.

Group / Team Leader Computational Biology or Bioinformatics

The Structural and Computational Biology Unit at EMBL Heidelberg, Germany, seeks to recruit an outstanding group leader or team leader in computational biology or bioinformatics working in areas such as structural bioinformatics (e.g. modeling of protein complexes and their interactions and/or dynamics in a cellular context), image analysis/visualisation (e.g. reading out data from GFP screens, E-tomograms or visualising a virtual cell atlas), cheminformatics (e.g. chemical-protein-network analysis), systems bioinformatics (e.g. tissue modeling, analysis network perturbations) or transcriptional regulation/epigenetics (e.g. chromatin modification analysis).

It is desirable that the new activities complement and are synergistic to existing research in the unit. The vision of the unit is to integrate the various structural biology approaches (Xray, NMR, electron microscopy, tomography and light microscopy) with computational, chemical and systems biology.

We are open to applications from candidates who wish to focus entirely on their own research projects and, because there is an increasing need to integrate and streamline the multiple computational approaches followed within EMBL, to candidates who wish to function at the interface between research and service activities.

The successful candidate should demonstrate a strong motivation to work in the multidisciplinary and collaborative environment of EMBL, reaching out to the many other computational and experimental groups.

Please apply online through www.embl.org/jobs and include a cover letter, CV and a concise description of research interests and future research plans. Please also arrange for 3 letters of recommendation to be emailed to references@embl.de at the latest by 6 May 2012.

Interviews are planned for 24 and 25 May 2012.

Further details on Team/Group Leader appointments can be found under www.embl.org/gl_faq.

For further Information about research in the Structural and Computational Biology Unit and at EMBL please visit ...

www.embl.org



The Philipps-Universität Marburg and the Max Planck Institute for Terrestrial Microbiology have recently established a center for Synthetic Microbiology, SYNMIKRO. Supported by the state of Hessen within its excellence program LOEWE, SYNMIKRO focuses on basic research in synthetic biology on all levels of microbial function, ranging from the development of regulatory circuits, genetic codes and metabolic pathways to the synthesis of new minimal cells and microbial communities.

In its next phase of expansion, SYNMIKRO invites applications for

Independent Research Group Leader positions (E15 TV-H) in the areas:

Quantitative analysis of metabolic networks

Development of synthetic circuits

Reprogramming of the genetic code

Molecular evolution, metagenomics and prokaryotic biodiversity

at the Philipps-Universität Marburg. We seek individuals with an outstanding track record in the listed research areas. Successful candidates are expected to pursue research projects connected to the activities of Synmikro as described at www.synmikro.de.

We provide attractive start-up packages and research funding, and offer teaching associations within the faculties of biology or chemistry, depending on the successful candidate's background.

The appointment is limited for a period of 4 years, with a 1-year extension after a positive evaluation in the third year and offers the opportunity for further scientific qualification - within the scope of the assigned tasks.

Informal inquiries can be directed to Prof. Dr. Bruno Eckhardt, Director SYNMIKRO, Philipps-Universität Marburg, director@synmikro.uni-marburg.de.

We support women and particularly invite them to apply. Applicants with children are welcome - the Philipps-University is certified as a family friendly university. Sharing a full-time position (§ 8 Abs. 2 Satz 1 HGlG) as well as a reduction of working time is possible. Applicants with a disability as described in SGB IX (§ 2 Abs. 2, 3) will be preferred in case of equal qualifications.

Please submit your application preferentially in one PDF file since application documents will not be returned.

Applications should be submitted by, April 10th, 2012 to info@synmikro.uni-marburg.de. The application should contain the following information in a single pdf-document: (1) cover letter, (2) curriculum vitae, including a list of publications, (3) summary of research achievements, (4) research plan, (5) statement of teaching interests and experience, if applicable, (6) previous and current funding, if applicable, (7) names and contact details of five referees.



Tenure-track Faculty Position (Assistant/Associate/Full Professor) Microbiome Initiative

The NYU Langone Medical Center is currently seeking scientists studying the role of human commensals (that comprise the microbiome) in host physiology. Investigators from diverse backgrounds (including but not limited to immunology, microbiology, physiology, biochemistry, pathology, genetics, genomics, chemistry, bioinformatics) are invited to apply for tenure-track positions that are a partnership between our innovative initiative in the Human Microbiome and our Departments of Medicine, Microbiology, and Pathology.

Our initiative aims to marry NYU Langone Medical Center's traditional strengths in infectious diseases and global health with its research on microbial pathogenesis, mechanisms and genetics of host-pathogen interactions, and adaptive and innate immunity. Our technological advantages in computational biology, model organisms, and assay development as applied to microbiota also enrich this exciting research environment.

Study of the human microbiota is one of the pillars of our academic trajectory, making us particularly interested in candidates focusing on analyzing the human microbiome in health and disease and the development of relevant animal models. Successful candidates will have the ability to strategically align available institutional and external resources to achieve their research goals.

Applications should include a *curriculum vitae*, a description of research accomplishments, plans for future research, and reprints of three representative publications. Applicants should also arrange to have three letters of recommendation submitted on their behalf. Application materials should be emailed as PDF files to: **HMP Search Committee, c/o Sandra Fiorelli (Sandra.Fiorelli@nyumc.org), NYU Langone Medical Center, 550 First Avenue, OBV-A606, New York, NY 10016.** The deadline for receipt of applications is **30 June 2012**.

Women and minorities are encouraged to apply. NYU is an Equal Opportunity/Affirmative Action Employer.



Smithsonian
National Museum of Natural History

SYSTEMATIC ENTOMOLOGIST

The Smithsonian's National Museum of Natural History seeks two systematic entomologists to conduct integrative, collections-based research programs focused on terrestrial arthropods or aquatic insects. Each successful candidate is expected to develop an internationally recognized research program utilizing modern methods, which may include bioinformatics, in pursuing systematic research on Diptera, Heteroptera, Coleoptera, or another terrestrial arthropod or aquatic insect group, with relevance to phylogenetics, genetics, evolution, morphology, behavior, biogeography, biodiversity, ecology, or related fields. Frequent publication of highly regarded papers in competitive, peer-reviewed journals, curation of collections in specialty area, service to the scientific community in leadership capacities, acquisition of external funding, engagement in outreach activities, and mentorship of students are expected.

There are two positions: (i) Federal Civil Service (U.S. citizenship required) and (ii) Trust (private sector, U.S. citizenship not required, proof of eligibility to work in the U.S. required). Both will be filled at the GS-12 level (\$74,872-\$79,864 per year). **Applicants who are U.S. citizens are encouraged to apply for both the Federal and Trust Funded positions (two applications required).** For application procedures go to www.sihr.si.edu and refer to Announcements **12A-RB-297507-DEU-NMNH (Federal)** or **12A-RB-297508-TRF-NMNH (Trust)**, or contact **Robinette Burrell, 202-633-6318, burrellro@si.edu**. Applications must be received online by **April 11, 2012** and must reference the announcement number. Applicants will be notified by email when their applications are received.

We encourage all qualified candidates to apply for both positions.

The Smithsonian Institution is an Equal Opportunity Employer.

Weill Cornell Medical College in Qatar

TYPE 2 DIABETES SENIOR INVESTIGATOR

BIOMEDICAL RESEARCH PROGRAM

FACULTY POSITION

Weill Cornell Medical College in Qatar (WCMC-Q), a branch of Weill Medical College of Cornell University, seeks a Type 2 Diabetes Senior Investigator to join its biomedical research program.

WCMC-Q is presently in its 10th year of operations and continues to pursue excellence in education, research, and clinical care. In a pioneering international initiative, the WCMC-Q research program was established in partnership with the Qatar Foundation for Education, Science and Community Development to address the most pressing health challenges in Qatar and the region by employing an integrated basic, translational and clinical approach.

WCMC-Q seeks a highly qualified candidate at the associate or full-professor level with an established research program in the cellular/molecular basis of Type 2 Diabetes. The successful candidate will have a stellar track record of research accomplishments and an enthusiasm for building new research initiatives. Candidates must have an MD and/or PhD degree and must be willing to relocate to Doha, which is rapidly growing as a research hub. The level of appointment will be commensurate with credentials and experience. A comprehensive and highly competitive salary and foreign service benefits package and a competitive start-up package will be provided.

The WCMC-Q research program offers a collaborative, multidisciplinary team environment, endowed with a comprehensive support infrastructure. The successful applicant will be able to draw on state-of-the-art facilities, including genomics, proteomics, imaging, and computational and biostatistics cores. Details regarding the WCMC-Q research program and facilities can be accessed at <http://qatar-weill.cornell.edu/research/index.html>.

Qualified applicants are invited to submit a letter of application, which should outline their interest in the position and a description of research interests and future research plans (3-5 pages), along with their curriculum vitae to:

<http://job.qatar-med.cornell.edu>

Applications may be submitted until June 30, 2012. Please note that, due to the high volume of applications, only short-listed candidates will be contacted.

Cornell University is an equal opportunity, affirmative action educator and employer.



Weill Cornell Medical College in Qatar



Diamond Light Source is a world leading, high brilliance synchrotron light source equipped with state-of-the-art experimental stations designed to enable scientists and engineers to undertake highly innovative research across a wide range of fields and with a wide variety of potential applications to industrial competitiveness and quality of life. We are currently seeking a new Chief Executive Officer to succeed Professor Gerhard Materlik in October 2012. Diamond is an energetic and dynamic organisation employing over 400 people from around the world. In addition over 2000 researchers use Diamond's world class beamlines every year to conduct experiments in a wide range of disciplines.

Chief Executive Officer

Diamond Light Source Ltd is a company jointly funded by the Science and Technology Facilities Council on behalf of the U.K. Government and the Wellcome Trust. As Diamond Chief Executive Officer you will be expected to provide overall strategic direction and lead the day-to-day management of the company as well as ensuring the continued smooth running and on-going operations of the synchrotron facility and the delivery of the Phase III suite of beamlines. You should have the necessary experience of synchrotron facilities and management of scientific laboratories in order to provide the appropriate level of leadership and strategic direction for the facility. You will be an inspirational leader, able to manage and motivate other people, and continue to attract high calibre staff from around the world to work at Diamond. With experience of managing complex projects and relationships you will be an excellent communicator with strong influencing and negotiating skills.

The post will be available from October 2012 and will be offered for a period of up to 7 years (with the possibility of extension). An internationally competitive remuneration package will be offered commensurate with the level of responsibility and is negotiable depending on experience.

For further information contact the Chair of the Diamond Search Committee, Sir Peter Williams on peter.williams@royalsociety.org
Details of the job description and the project are also available on the website: www.diamond.ac.uk

For an informal confidential discussion with the current Chief Executive Officer on the role please contact his PA, Ms Karen Habgood on 01235 778445 or email to Karen.habgood@diamond.ac.uk

Written applications, including a curriculum vitae and the details of three referees, should be sent to recruitment@diamond.ac.uk
Applications should be received by 27 April 2012.

Interviews will be held in the week commencing 18 June 2012.

References may be sought in advance of interview.

Permission will be sought prior to seeking references

www.diamond.ac.uk





Introductory Course Instructor and Manager Department of Physics

The Department of Physics seeks applicants for the position of Introductory Course Instructor and Manager. This individual will be responsible for teaching algebra- and calculus-based introductory physics courses and coordinating the delivery of several large enrollment physics courses offered every semester. Specific duties and responsibilities include:

- Lecturing, teaching laboratory or discussion sections in large calculus- or algebra-based physics courses.
- Work with a large course instructional team which will include 2 or 3 faculty members.
- Instruct, oversee and coordinate the activities of graduate teaching assistants.
- Develop, produce, and administer exams.
- Develop and produce new course material.
- Work with instructional support team to insure that materials are available for students as needed.
- Work with faculty in all large courses to insure that delivery of material and course policies are uniform.
- Work with the Director of Course Delivery in educating faculty on how to use the online tools available for their courses.
- Establish and direct a program to mentor and monitor teaching assistants.
- Oversee scheduling of office hours and review sessions for all large courses.
- Assist faculty and staff in handling issues regarding student enrollments and grades.
- Work with staff in the Undergraduate Office to improve efficiency and develop best practices.

Candidates must possess an advanced degree (M.S. or Ph.D.) in Physics or a closely related field and have significant classroom teaching experience. Knowledge of web-based programming applications is preferred, but not required. The successful candidate is expected to be passionate about teaching and working closely with students. The candidate must work well as part of a teaching team and is expected to work well with faculty, administrative professionals and staff. Strong communication, organizational, planning and management skills are required. An ability to work with diverse domestic and international student constituencies is also necessary.

The Introductory Course Instructor and Manager position is a full-time, benefits-eligible academic position appointed on a 12-month service basis. The anticipated start date is August 16, 2012, or as soon as possible after the closing date. Applicants may be interviewed before the closing date; however, no hiring decision will be made until after that date. Salary is commensurate with experience and qualifications.

To apply for this position, please create your candidate profile at <http://jobs.illinois.edu> and upload your cover letter, resume, and names/contact information for three references by **April 18, 2012**. Full consideration will be given to complete applications received by the closing date. For further information regarding application procedures, contact **Margie Gamel** at 217-333-3762 or mgamel@illinois.edu.

*Illinois is an Affirmative Action/Equal Opportunity Employer and welcomes individuals with diverse backgrounds, experiences, and ideas who embrace and value diversity and inclusivity.
(www.inclusiveillinois.illinois.edu)*



Florida State University College of Medicine Tenure-track Faculty Position for a Microbiologist

The Department of Biomedical Sciences at the Florida State University College of Medicine invites applications for a tenure-track faculty position for a microbiologist at Assistant/Associate/Full professor level. The applicant will hold a PhD or MD/PhD with a demonstrated record of success in research and potential for excellence in teaching. Established investigators will be expected to have extramurally supported research, and early-stage investigators will be expected to develop and sustain an extramurally funded research program. Applicants should submit a letter of application, curriculum vitae, an overview of future research plans, a statement of teaching interest and/or experience with medical students, and a list of at least three references in a single pdf file to biomedfacultysearch@med.fsu.edu.

The College of Medicine has recently invested in a translational science core laboratory equipped with new mass spectrometry and deep sequencing equipment. The Department of Biomedical Sciences has more than 150,000 sq. ft. of new, state-of-the-art laboratory space, core labs in proteomics, genomics, confocal and spinning disk-microscopy, flow cytometry, and cell culture. Current research in the department is broadly focused on understanding the molecular bases of human diseases and regenerative medicine. At FSU, collaborative opportunities abound within the Department of Biomedical Sciences, in the departments of Biological Sciences, Chemistry and Biochemistry, Computational Science, Psychology, in the Institute for Molecular Biophysics, and at the National High Magnetic Field Laboratory. See <http://www.med.fsu.edu/?page=biomedicalsciences> for more information.

In 2011, the FSU College of Medicine received an 8-year accreditation from the Liaison Committee for Medical Education (LCME) for its medical education program. The college has a history of producing outstanding graduates who are accepted into excellent residency training programs, have high passage rates on the United States Medical Licensing Exam (USMLE), and who are trained to practice patient-centered health care.

*The Florida State University is an Equal Opportunity/
Affirmative Action Employer.*

Director of Research (DoR)



The Office of Naval Research (ONR) within the Department of Navy announces an Intergovernmental Personnel Act (IPA) recruitment for the Director of Research. ONR is the science and technology (S&T) provider for the Department of the Navy, advancing the operational concepts and visions for the Navy and Marine Corps of the future. ONR plays a critical role in advancing scientific knowledge to support the next generation of naval technologies with a vision focused on future capabilities and by hedging against uncertainty of warfare. The agency coordinates, executes, and promotes the science and technology programs of the United States Navy and Marine Corps through schools, universities, government laboratories, and nonprofit and for-profit organizations.

The Director of Research (DoR) is responsible to the Chief of Naval Research for the overall integration of the Discovery and Invention (D&I) S&T portfolio in support of naval needs. The objective of the about \$900M D&I S&T portfolio is to make broad investments in basic and applied research that increase fundamental knowledge, foster opportunities for breakthroughs and provide technology options for future Naval capabilities and systems. The DoR provides recommendations to ONR leadership on the quality and balance of the various investment areas, through consideration of naval needs, technology trends, defense guidance, and resources.

For information regarding this vacancy and specific instructions on how to apply, go to <http://onr.navy.mil>. Please carefully read the job description and follow instructions when applying. **The deadline for submission is 04/30/2012.** For further information please contact Valeria Payne at valeria.payne@navy.mil.

For technical questions regarding this announcement, please contact:

CAPT Doug Marble
Assistant Chief of Naval Research
douglas.marble@navy.mil
703-696-8459

Dr. Kam Ng
Deputy Director of Research
kam.ng@navy.mil
703-696-0812

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IN THE WORKPLACE. WOMEN AND MINORITIES ARE ENCOURAGED TO APPLY.**



Weill Cornell Medical College in Qatar

POSTDOCTORAL POSITION

In a pioneering international initiative, Cornell University and Weill Cornell Medical College established the Weill Cornell Medical College in Qatar (WCMC-Q) through a unique partnership with the Qatar Foundation for Education, Science and Community Development. Located in Doha, Qatar, and in its tenth year of operation, WCMC-Q seeks candidates for a postdoctoral associate position in Doha in:

NEXTGEN SEQUENCE ANALYSIS

The Bioinformatics Core and Suhre Lab (<http://qatar-weill.cornell.edu/research/laboratories/bioinformatics.html>) at WCMC-Q invites applications for a postdoctoral associate position in the area of next generation sequence analysis and bioinformatics. The candidate will investigate the genetic basis of monogenic as well as of complex disorders that are relevant to the Middle East population. With the advent of cheap whole genome sequencing, translating huge genome data sets into clinically relevant applications is a major challenge. The successful applicant will identify and apply adequate computational tools from the NextGen field to original biological data generated at WCMC-Q's genomics core. De-novo software development will be limited. Focus is on the biological interpretation of the computational results in a clinical relevant setting.

Candidates must hold a Ph.D. degree, ideally in computational biology or bioinformatics. Applicants with a strong background in scientific computing from unrelated fields (e.g. high-energy physics), but who are willing to train in genomics, will also be considered. Proficiency in Linux scripting and one major programming language are a must.

WCMC-Q is located in Education City among other American universities in Doha, a rapidly growing research hub. The WCMC-Q research program offers a collaborative, multidisciplinary team environment, endowed with a comprehensive support infrastructure. Details regarding the WCMC-Q research program and facilities can be accessed at <http://qatar-weill.cornell.edu/research/index.html>.

WCMC-Q postdoctoral associates and research associates are appointed by the academic departments at Weill Cornell Medical College. Salary is commensurate with experience and is accompanied by an attractive foreign-service benefits package, including paid housing and a car allowance.

Qualified applicants are invited to submit a letter of application, which should outline their interest in the position, along with their curriculum vitae including the names and contact details of three referees to:

<http://job.qatar-med.cornell.edu>

Positions are open until filled. Please note that, due to the high volume of applications, only short-listed candidates will be contacted.

Cornell University is an equal opportunity, affirmative action educator and employer.

Technische Universität München



The **MUNICH CENTER FOR TECHNOLOGY IN SOCIETY (MCTS)** is a new cross-faculty Integrative Research Center which is currently being developed at Technische Universität München (TUM). It concentrates in both research and teaching on the interactions among the sciences, technology and society. The intention is to add a societal dimension to research at TUM—in foundational and applied research as well as in the implementation of new technologies. The **MCTS** invites applications for the position of

Professor of Sociology of Science

(Friedrich Schiedel endowed chair, pay grade W3 corresponding to full professor) to be filled as soon as possible.

The Technische Universität München is committed to excellence in research and teaching. We seek a researcher with an outstanding international track record who represents the sociology of science and technology in the MCTS research focus Science and Technology Studies (STS). The successful candidate is expected to work closely with his/her colleagues in the STS area including the already existing professorships (e.g. Philosophy of Science, History of Technology, Science Management) and others that have yet to be established (e.g. Science Policy, Innovation Research). To ensure interaction between the MCTS as center for social sciences and humanities with the traditional disciplines at TUM, the professorship will be anchored as a joint appointment in one of the regular faculties depending on the scientific profile of the successful candidate. To guarantee excellent international research, close cooperation with the TUM Institute of Advanced Study is expected. Also, collaboration with the scientific partners of MCTS on the regional, national and international level is desirable.

Applicants should demonstrate exceptional pedagogical skills along with a strong commitment to teaching both in the development of new degree programs at MCTS and in courses for students of faculties that are associated with MCTS.

Requirements for the employment of professors at universities in Bavaria apply (Art. 7 and 10 Abs. 3 BayHSchPG).

As part of the excellence initiative of the German federal and state governments, the Technische Universität München has been pursuing the strategic goal of substantially increasing the proportion of women in research and teaching. Thus, female scientists are explicitly encouraged to apply for this position.

The TUM Munich Dual Career Office provides support for dual career couples and families.

Preference will be given to disabled candidates with essentially the same qualifications.

Applications accompanied by supporting documentation (CV, certificates, list of publications including selected reprints, list of courses taught, and a statement of research and teaching goals and strategies) should be submitted by **30. April 2012** to:

Prof. Dr. Klaus Mainzer

Director, MUNICH CENTER FOR TECHNOLOGY IN SOCIETY
c/o Carl von Linde-Akademie
Technische Universität München
Arcisstraße 21
80333 München
Germany

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Science Careers

From the journal *Science*



ScienceCareers.org



The Philipps-Universität Marburg and the Max Planck Institute for Terrestrial Microbiology have recently established a center for Synthetic Microbiology, SYNMIKRO. Supported by the state of Hessen within its excellence program LOEWE, SYNMIKRO focuses on basic research in synthetic biology on all levels of microbial function, ranging from the development of regulatory circuits, genetic codes and metabolic pathways to the synthesis of new minimal cells and microbial communities.

In its next phase of expansion, SYNMIKRO invites applications for an

Independent Research Group Leader position (E15 TV-H) in the areas: Computational Biology / Mathematical modeling of microbial systems

at the Philipps-Universität Marburg. We seek a person with an outstanding track record in the quantitative analysis and modeling of biological systems. Successful candidates are expected to pursue research projects connected to the activities of Synmikro as described at www.synmikro.de. Experimental work will be encouraged and opportunities will be offered.

We provide an attractive start-up package and research funding, and offer teaching associations with the faculties of mathematics and computer science, biology, or physics, depending on the successful candidate's background.

The appointment is limited for a period of 4 years, with a 1-year extension after a positive evaluation in the third year and offers the opportunity for further scientific qualification - within the scope of the assigned tasks.

Informal inquiries can be directed to Prof. Dr. Bruno Eckhardt, Director SYNMIKRO, Philipps-Universität Marburg, director@synmikro.uni-marburg.de.

We support women and particularly invite them to apply. Applicants with children are welcome - the Philipps-University is certified as a family friendly university. Sharing a full-time position (§ 8 Abs. 2 Satz 1 HGLG) as well as a reduction of working time is possible. Applicants with a disability as described in SGB IX (§ 2 Abs. 2, 3) will be preferred in case of equal qualifications.

Please submit your application preferentially in one PDF file since application documents will not be returned.

Applications should be submitted by, April 10th, 2012 to info@synmikro.uni-marburg.de. The application should contain the following information in a single pdf document: (1) cover letter, (2) curriculum vitae, including a list of publications, (3) summary of research achievements, (4) research plan, (5) statement of teaching interests and experience, if applicable, (6) previous and current funding, if applicable, (7) names and contact details of five referees.

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Science Careers

From the journal *Science*



ELAN PHARMACEUTICALS
LEADING INNOVATION



Elan is a neuroscience-focused biotechnology company that is committed to discovering and developing therapeutics aimed at changing the underlying mechanisms of neurodegenerative disease. We are committed to innovative science to address significant unmet medical needs for patients, their families and clinicians.

Our Research and Development scientists in South San Francisco are at the forefront of research for Parkinson's disease, Alzheimer's disease and multiple sclerosis, and are aggressively pursuing new therapeutic approaches by leveraging our depth in neuroscience, immunology, and our cutting-edge collaborations around the world.

We are dedicated to having the best people and the best resources work together to move our science forward for patients. Currently we have multiple opportunities for post-doctoral candidates and scientists in the following areas:



- Biochemistry - Immunology - Translational Science
- Structural Biology - Pharmacology - Assay Development
- Neuropathology - Antibody Discovery - SPR Screening

To obtain more information and apply, please visit the Elan website:
www.elan.com



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POSITIONS OPEN



University College Dublin and Elan Corporation plc

Interdisciplinary Chair in the Business of Biotechnology Permanent post – Ref: 005025

University College Dublin and Elan Corporation plc, are establishing Europe's first interdisciplinary Chair in the 'Business of Biotechnology' which will sit at the intersection of the UCD Michael Smurfit Graduate Business School and the UCD College of Science.

The *Professor of the Business of Biotechnology* will spearhead the education of a new breed of biotechnology entrepreneurs combining the study and application of business management principles and practices with advancements in biology, computational science, diagnostics and therapeutic treatments. The Chair will lead in establishing and sustaining an international track record in the field of integrated biotechnology innovations in management and science.

UCD seeks to appoint an internationally renowned academic who will provide key leadership at the interface between science and business management. For further information, including details on how to apply, please visit www.ucd.ie/jobvacancies

Closing date: 23:30 BST on Sunday 22nd April 2012

UCD is an equal opportunities employer

About UCD

University College Dublin is one of Europe's leading research-intensive universities, with 38 Schools and over 24,000 students. Establishing, supporting and sustaining this Chair is a key priority for UCD and an integral element of the University's strategic plan: *Forming Global Minds*.

For additional information about UCD, please visit www.ucd.ie

About Elan

Elan is a neuroscience focused biotechnology company committed to making a difference in the lives of patients and their families by dedicating itself to bringing innovations in science to fill significant unmet medical needs that continue to exist around the world. For additional information about Elan, please visit www.elan.com



Extraordinary Possibilities

AWARDS



HUMAN FRONTIER SCIENCE PROGRAM (HFSP)

CALL FOR NOMINATIONS FOR THE 2013 HFSP NAKASONE AWARD

In keeping with its mission to stimulate innovative international research, HFSP invites nominations for an annual award highlighting ground-breaking contributions in the life sciences. Typically these will be breakthroughs in understanding the complex mechanisms of living organisms that have important consequences for scientists throughout the world. Experimental, conceptual and technological contributions are eligible. This award recognizes the vision of former Prime Minister Nakasone of Japan in the creation of HFSP.

The winner of the 2012 award was Gina Turrigiano of Brandeis University, USA, for her pioneering work on homeostatic plasticity in the brain.

The competition is open; it is not limited to HFSP awardees and there is no age limit for candidates. However the jury will pay particular attention to recent breakthroughs by younger scientists. Nominations should be made before Friday, May 4th by submitting the standard one-page nomination form and the nominee's CV (see <http://bit.ly/e2hSIs> for more information). The selection will be made by the HFSP Council of Scientists at their meeting in July 2012.

The awardee will receive an unrestricted research grant of 10,000 USD, a commemorative medal and will be expected to deliver a plenary lecture at the 2013 HFSP Awardees Meeting, July 8-10 2013 in Strasbourg, France.

HFSP, 12 quai Saint-Jean, 67080 STRASBOURG Cedex, France,
www.hfsp.org

POSITIONS OPEN



Department of Health and Human Services National Institutes of Health National Library of Medicine Chief, Office of International Programs

The National Library of Medicine (NLM), a major component of the National Institutes of Health (NIH), is seeking exceptional candidates for the position of Chief of the Office of International Programs. As the world's largest medical library, the NLM is responsible for collecting, preserving, and promoting the dissemination of information important to the progress of medicine and public health, both nationally and internationally. The Chief of the Office of International Programs coordinates and conducts efforts to foster the understanding, access, and use of NLM's resources around the world, with particular attention to developing countries.

The successful candidate for this position will serve as the NLM expert, point of contact, and liaison to other organizations for international and global health. This position is responsible for providing expert information in this area to the leadership and senior staff of the NLM, and for consulting with other staff across NIH. In addition, the incumbent helps lead the development of policy in the area of international and global health as it relates to the mission of NLM, and coordinates and manages bilateral and multilateral efforts between and among NLM and foreign partners. The Chief of the Office of International Programs also works with other government agencies and private organizations to pursue the mission of NLM around the world. The duties of this position also include the management of several ongoing activities with librarians, health care providers and scientists in sub-Saharan Africa. Key competencies for this position include expertise in international and global health, including: the ability to work with organizations and represent the interests of the NLM, ability to understand and help develop relevant science policy, ability to analyze and evaluate relevant research programs, and the ability to communicate complex information to a variety of audiences. For additional information, and instructions on applying to this position, please visit <http://www.usajobs.gov/> and search for announcement numbers: NIH-NLM-DE-12-620064 or NIH-NLM-MP-12-6620420. All applications must be received via the automated system to be considered.

DHHS, NIH, and NLM are Equal Opportunity Employers.

PRIZES



The 2012 (28th)
International
Prize for Biology



Calling for Nominations

This year's Research field: **Neurobiology**

Please access to: <http://www.jsps.go.jp/english/e-biol>

Deadline: May 11, 2012

• The International Prize for Biology was instituted in April of 1985 by the Committee on the International Prize for Biology in commemoration of the sixty-year reign of Emperor Showa and his longtime devotion to biological research.

• To commemorate the 25th anniversary of the institution of the Prize in 2005, it has been decided to also offer tribute to the present Emperor His Majesty Emperor Akihito, who has strived over many years to advance the study of taxonomy of global fishes while contributing continuously to the development of this Prize.

• The Prize is awarded each year to an individual who has made an outstanding contribution to the advancement of basic research in a field of biology.

• This year, the applicable area of the Prize was stipulated as 'Neurobiology'.

Recent years Prize Winners

2011. Dr. Eric Harris Davidson (Developmental Biology)
2010. Dr. Nancy Ann Moran (Biology of Symbiosis)
2009. Dr. Winslow Russell Briggs (Biology of Sensing)

POSITIONS OPEN



POSTDOCTORAL POSITIONS on Genomic Medicine

Two NIH-funded postdoctoral positions are available in the Department of Pharmaceutical Sciences at the University of Connecticut School of Pharmacy to study pharmacogenomics, pharmacopigenomics, and personalized medicine. Recent Ph.D. graduates with a background in pharmacology, drug metabolism, genetics, epigenetics, genomics, RNA biology, or bioinformatics are encouraged to apply. Please send a cover letter, curriculum vitae, and the names of three references and their contact info to **Leslie LeBel** (e-mail: leslie.lebel@uconn.edu).

The University of Connecticut is an Equal Employment Opportunity/Affirmative Action Employer.

ASSOCIATE OR FULL PROFESSOR Department of Anesthesiology University of Florida

The Department of Anesthesiology at the University of Florida is recruiting for a prominent Ph.D. neuroscientist for a tenure or non-tenure-track Associate or Full Professor. Applicants should currently have significant federal funding, including being Principal Investigator on one or more NIH grants of the R01, P or U series that complement existing clinical interests in chronic pain. Focus areas may include changes in ion channels leading to congenital analgesia, channel mutations resulting in extreme pain disorders, other electrophysiological disorders, etc. Via these skill sets, the successful applicants will directly participate and foster neuro-related basic and clinical research, a strategic area of interest to the College of Medicine and University. In addition, the applicant should be integrated into the national network of pain researchers studying these phenomena. The anticipated start date of this position is July 1, 2012. Interested individuals should respond by April 29, 2012, please send your curriculum vitae to: **Mary Ann Hoyt, Office Manager, Department of Anesthesiology, PO Box 100254, Gainesville FL 32610-0254** or to e-mail: mhoyt@anest.ufl.edu. *An Equal Opportunity Institution.*

Nature Neuroscience seeks an **ASSISTANT EDITOR**. The journal publishes high-quality papers in all areas of neuroscience and provides a highly visible forum for communicating important advances to a broad readership. For more information about the journal, see our website: <http://www.nature.com/natureneuroscience>.

Applicants must have a Ph.D., a strong research background (in any area of neuroscience), broad interest in neuroscience, excellent literary skills, commitment to the communication of scientific ideas, and willingness and ability to learn new fields.

The candidate will participate in all aspects of the editorial process; commissioning, editing News & Views, and writing Reviews for the journal. The job also involves attending meetings in the U.S. and abroad to maintain contact with the international scientific community.

Position located in New York City and resides in the Nature Publishing Group of Research journals. International and domestic travel is required to maintain contact with the scientific community.

To apply, go to website: <https://home.eease.adp.com/recruit/?id=56966>.

Equal Opportunity Employer. Website: <http://www.nature.com>.

POSITIONS OPEN

ASSISTANT, ASSOCIATE, OR FULL Professor Faculty Molecular or Cellular Infectious Diseases Research

The Division of Allergy and Infectious Diseases in the Department of Medicine and the Center for Emerging and Reemerging Diseases (CERID) at the University of Washington is recruiting for three full-time faculty positions in either the research or regular faculty track, without tenure at the rank of Assistant, Associate, or Full Professor. CERID's mission is to facilitate synergistic infectious diseases research excellence in emerging and re-emerging infectious diseases, with specific strengths in host defense, biochemistry, immunology, pathogenesis, and drug & vaccine development.

The successful candidate's research will focus on molecular and/or cellular infectious diseases research. These positions are designed for faculty with a basic science or translational research program with independent grant funding that focuses on microbial pathogenesis, therapy, vaccines, or the host immune response. These candidates must hold a doctoral level degree (M.D., Ph.D., or M.D.-Ph.D.) in disciplines such as infectious disease, immunology, microbiology, biochemistry, molecular biology, cell biology, or systems biology. M.D. candidates would serve as a core physician/scientist and/or educator, in the areas of medicine mentioned above. In order to be eligible for University sponsorship for an H-1B visa, graduates of foreign (non-U.S.) medical schools must show successful completion of all three steps of the U.S. Medical Licensing Exam (USMLE), or equivalent as determined by the Secretary of Health and Human Services.

University of Washington faculty engage in teaching, research, and service. Interested parties should electronically send their curriculum vitae and letter of interest to: **Thomas Hawn, M.D., Ph.D., Associate Professor of Medicine, University of Washington, 1959 NE Pacific Street, Mailbox 356423, Seattle, WA 98195-6423, e-mail: jgordon@medicine.washington.edu**. These positions are open until filled. *The University of Washington is an Affirmative Action/Equal Opportunity Employer. The University is building a culturally diverse faculty and staff and strongly encourages applications from women, minorities, individuals with disabilities and protected veterans.*

POSTDOCTORAL FELLOW POSITION in Neuroscience

A postdoctoral position is open immediately in the Department of Neuroscience of the Albert Einstein College of Medicine. The research project investigates cellular mechanisms regulating neuronal excitability and synaptic transmission in the striatum of a mouse model of Huntington's disease. Experience with electrophysiological recording techniques is preferred. The Department of Neuroscience has an active research and training program, and collaboration with other groups is definitely encouraged. Applicants should send curriculum vitae and a description of their research experience to: **Dr. Donald Faber, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Kennedy Center 429, Bronx, NY 10461. E-mail: donald.faber@einstein.yu.edu**. They should also arrange for three letters of reference to be sent to the address above. *Equal Opportunity Employer.*

FACULTY POSITIONS Medical School

The Saint James School of Medicine, an international medical school (website: <http://www.sjsm.org>), invites applications from candidates with teaching and/or research experience in any of the basic medical sciences for its Caribbean campuses. Senior faculty positions are currently available in Pathology. Applicants must be M.D., D.O., and/or Ph.D.

Teaching experience in the U.S. system is desirable but not required. Retired persons are encouraged to apply. Attractive salary and benefits. Submit curriculum vitae electronically to e-mail: mjansen@mail.sjsm.org or mail to: **HRDS Inc., 1480 Renaissance Drive, Suite 300, Park Ridge, IL 60068.**

POSITIONS OPEN

GENOMICS DIRECTOR

The Center for Genomics and Bioinformatics (CGB) at Indiana University (website: <http://cgb.indiana.edu>) is currently accepting applications for a Genomics Director, who oversees the genomics laboratory (including next generation sequencing and Nimblegen microarray platforms) and its core personnel. The Director hires and trains core staff, operates training workshops, and acts as a catalyst for interactions between the CGB and IU faculty. S/he has many collaborative research opportunities.

We seek an individual with excellent communication skills, research interests in genomics/bioinformatics, and a strong postdoctoral track record. The Genomics Director will be appointed to the University's research faculty with rank and remuneration depending upon experience. Inquiries about all the openings should be directed to e-mail: jobs@cgb.indiana.edu.

The Genomics Director position is available immediately. Applications received by May 1, 2012 will be assured full consideration. To apply, please submit curriculum vitae and a description of your background and interests, and arrange that three letters of recommendation be sent directly to:

**Genomics Director Search
Center for Genomics and Bioinformatics
Indiana University 1001 E. 3rd Street
Bloomington IN 47405-3700**

Indiana University is an Affirmative Action Equal Opportunity Employer.

YALE UNIVERSITY Department of Chemistry

The Department of Chemistry at Yale University invites applications for the position of **LECTURER** or **SENIOR LECTURER** to commence August 1, 2012. We seek creative, inspirational educators who will be able to assume a central role in the department's general chemistry and laboratory teaching. Highly experienced teachers as well as recent Ph.D.s are encouraged to apply. Applicants should send their curriculum vitae, a statement of STEM teaching philosophy, and arrange for the submission of two letters of recommendation.

Please submit all material to Academic Jobs Online at website: <https://academicjobsonline.org/ajo/Yale> (Yale-CHEM-LECTURER #1485)

A review of applications will begin May 1, 2012. This search remains subject to final university approval. *Yale University is an Equal Opportunity/Affirmative Action Employer and applications from women and underrepresented minority group members are especially encouraged.*

POSTDOCTORAL RESEARCHER Bacterial Pathogenesis

A postdoctoral fellow position is immediately available to study molecular mechanisms of streptococcal pathogenesis. The ideal candidate should have a Ph.D. degree in molecular biology, biochemistry, cellular microbiology, microbial pathogenesis, or a related field with good publication record and communication ability. Experience working with animals is desired. Selected individual will be primarily responsible for conducting independent research on Gram-positive pathogens. Interested individuals should send curriculum vitae with names and addresses of three references to: **Vijay Pancholi, Ph.D. Associate Professor, Department of Pathology, College of Medicine at The Ohio State University, TMRF-288 420 W 12th Avenue, Columbus, OH 43210-1214, Telephone: 614-688-8053, e-mail: vijay.pancholi@osumc.edu.**

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